FROM THE PÆDIATRIC CLINIC OF THE UNIVERSITY HOSPITAL: UPPSALA

STUDIES ON THE PLASMA TRANSPORT OF IRON

I. The Iron-binding Capacity of Serum in Infants and Children

II. Intravenous Iron, its Plasma Transport, Toxic Reactions, and Use in Pædiatrics

By

BENGT HAGBERG

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AKADEMISK AVHANDLING

som med vederbörligt tillstånd av Medicinska Fakulteten i Uppsala för vinnande av medicine doktorsgrad framlägges till offentlig granskning å Patologiska Institutionens hörsal lördagen den 25 april 1953 kl. 10 f. m.

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UPPSALA 1953
ALMQVIST & WIKSELLS BOKTRYCKERI AB

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INTRODUCTION

The available, non-haemoglobin iron in the body is attached to special proteins with iron-combining properties. The knowledge of their presence, nature, and function dates mainly from the years 1945—1950.

Iron is stored in an easily available form as ferritin, which consists of the protein apoferritin with attached iron. Normally this complex does not appear in the blood, where a special iron-binding plasma protein is to be found instead. It is called transferrin (siderophilin, beta$_1$-metal-combining globulin). The small but important amount of non-haemoglobin iron in the blood, the plasma or serum iron, is combined with this protein for transport in the blood. Only about one third of the total iron-binding capacity of the transferrin is, however, saturated at ordinary serum iron levels. The rest exists in an iron-free, unsaturated form. The concentrations of serum iron and transferrin and their mutual relationship become altered in characteristic ways in physiological and pathological changes in the iron balance. They are probably closely associated with the distribution, storage, and mobilization, of iron. Combined determinations of the two factors will therefore give a far better understanding of the actual state of the iron metabolism than measurement of the serum iron alone.

The first part of this work consists of a survey of the physiology of iron from the fetus to the adult. An account is then given of various changes in the serum iron and the iron-binding capacity of infants and children in health, iron deficiency, and infections.

The second part refers mainly to investigations on intravenous iron (saccharated iron oxide), i.e. its quantitative estimation in human serum, its plasma transport, its side-effects and their pathogenesis, the therapeutic effect, and finally the indications for its use in paediatrics.

These two main parts at first sight seem to have little in common. The theoretical basis for intravenous iron therapy with saccharated iron oxide is, however, intimately related to the above-mentioned mechanism of the transport of iron in plasma. This mechanism explains among other things why the human body tolerates large doses and huge plasma concentrations of colloidally bound iron, whereas much smaller quantities of simple iron salts give immediate toxic reactions.

Abbreviations

TIBC = Total iron-binding capacity.
UIBC = Unsaturated iron-binding capacity.
SI = Serum iron.
ESR = Erythrocyte sedimentation rate (micro-method).
RES = Reticulo-endothelial system.

I

The Iron-binding Capacity of Serum in Infants and Children

Also published as supplement 93 to Acta Pædiatrica

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CHAPTER I

General Review of Iron Metabolism

Although the total amount of iron in the body is extremely small, the fact that it is an essential constituent of haemoglobin, myoglobin, and certain enzymes, makes it an element of vital importance in metabolic processes. Its chief functions are concerned with the transport of oxygen to the tissues, and cellular respiration.

1. Distribution of iron in the body

The total iron in the body of an adult man amounts to 4—5 g. Not less than about 60 per cent consists of haemoglobin iron, about 5—10 per cent is found in the form of myoglobin, and of the rest 20—25 per cent is storage iron easily available for haemoglobin production. Less than one per cent is found in the extremely important enzymes cytochromes, cytochrome oxidase, peroxidase, and catalase. The remainder is practically all fixed cellular iron with but little known function. The small quantity of transport iron, the plasma iron, represents about 0.1 per cent of the total body iron (for references see Hemmeler, 1951; Wintrobe, 1952; Widdowson *et al.*, 1951; Finch *et al.*, 1950; Haskins *et al.*, 1952).

2. Ferritin and haemosiderin, and the storage of iron

The term 'storage iron' means the iron which can be mobilized as required from different tissues for the formation of haemoglobin. Ordinarily the storage iron is not utilized, but when production of red cells exceeds destruction, iron is immediately mobilized from the depots. On the other hand excess iron will be transported to the depots when the destruction of red cells increases in comparison with the new production. The iron is stored intracellularly in iron-protein complexes, mainly as ferritin, but to some extent also as haemosiderin.

Ferritin (Laufberger, 1937; Granick, 1942—1951) consists of a protein, apoferritin (mol. wt. 460000), and up to 23 per cent of iron. The colourless apoferritin is synthesized by the cells only when needed. It does not usually appear in free form. The iron is physically bound to apoferritin as ferric hydrox-

ide 'micelles' or 'clusters' (GRANICK, 1946). Ferritin can be produced in various cells and organs. Its formation seems to be the defence mechanism against an excess of free iron ions, and also the physiological means of storing iron in the body. Iron as ferritin is easily available and easily soluble, because apoferritin apparently constitutes a protective colloid for the low-molecular ferric hydroxide micelles. These are prevented from subsequently condensing and enlarging, and thus from precipitating in the form of an insoluble ferric hydroxide gel.

Haemosiderin has been regarded as an abnormal stage of iron deposition in the cells (GRANICK, 1949; SCHWIETZER, 1951). Another opinion has been expressed by FINCH and associates (1950), and others, who hold that the formation of haemosiderin is a second physiological form of iron storage. It is, however, mainly resorted to when there is excessive supply of iron to the depots, the formation of apoferritin being thought to be insufficient for transformation of large amounts of iron into ferritin. Haemosiderin, which contains less protein and more iron, is then formed. When its iron content increases the haemosiderin is thought to become more stable and insoluble. Though ferritin-iron is thus presumed to be more readily available than haemosiderin-iron, both become depleted from the tissues when iron is needed for erythropoiesis (FINCH *et al.*, 1950).

The liver contains most of the iron stored in the body. A smaller part of this iron fraction is found in the spleen and the bone marrow. Recently HASKINS *et al.*, 1952, have shown, by studying erythropoiesis following repeated phlebotomy in four men, that in all 1200—1500 mg of iron was available for the formation of haemoglobin. The depleted stores of iron were shown to be replaced very slowly. When no extra iron was given these were not even adequately filled one year later. In the replenishment of the iron depots it seems to be unimportant whether iron is administered by the oral or the parenteral route, as the ultimate distribution is found to be practically the same (FINCH *et al.*, 1950).

3. Excretion and absorption of iron

It is almost impossible to eliminate physiologically iron once incorporated in the body (MCCANCE & WIDDOWSON, 1937, 1938; BARER & FOWLER, 1937; HAHN *et al.*, 1939; and others). In the normal adult man an average loss of about 1 mg per day can be reckoned with, in the normal adult woman 2—3 mg depending on the menstrual loss and the frequency of pregnancies. The diurnal urinary excretion of iron is about $\frac{1}{2}$ mg, although many times as much iron passes through the kidneys during the day. Minute quantities are lost in the bile (GREENBERG *et al.*, 1943; HAWKINS & HAHN, 1944), and almost in-

significant amounts in shed intestinal and dermal cells. Evidently there is no colonic excretion of iron (McCance & Widdowson, 1943).

The mechanism of iron absorption seems to be complicated, and is only partially known. Iron in the food occurs usually as colloidal ferric hydroxide, and is reduced in the stomach to ferrous iron. The iron is better absorbed in this divalent than in trivalent form, as has been demonstrated in studies with radio-active iron (Moore *et al.*, 1944; Hahn *et al.*, 1945). Investigations using auto-radiography have definitely proved that most of the absorption occurs in the duodenum near to the pylorus (Endicott *et al.*, 1949). Iron can also be absorbed from the stomach (Vahlquist *et al.*, 1945) and from more distal parts of the intestine (Granick, 1946). Above all, absorption is favoured by a diet with a low content of phosphates (Gillman & Gillman, 1947; Hegsted *et al.*, 1949), with an excess of ascorbic acid (Moore & Dubach, 1951; and others), by the addition of copper to the diet (Chase, *et al.*, 1952), and by a deficiency of protein (Schwietzer, 1951).

The amounts of iron absorbed daily under physiological conditions are small. Preliminary studies with radio-active iron incorporated in normal foods have shown that commonly less than 10 per cent of the iron in the food is absorbed (Moore & Dubach, 1951). Thus in order to maintain iron-balance 10—30 mg iron per day must be introduced into the intestinal tract with the food. The results of the same investigation also suggest that iron-deficient subjects can absorb no more iron from the food than healthy persons. On the other hand, inorganic iron preparations are better absorbed by subjects with an increased demand for iron (Hahn *et al.*, 1943, 1951; and others). A surplus of iron in sufficient amounts can also increase the absorption in healthy individuals to some extent, but the greater the dose the smaller the proportion absorbed. Some regulative mechanism must be present, particularly as the body is practically unable to eliminate iron, i.e. to regulate the iron balance by excretion.

The saturation of the iron depots is considered to be the essential factor in the control of iron absorption (Balfour *et al.*, 1942), but the mechanism is still obscure. One suggestion is that control may take place through a disturbed equilibrium of the iron ion activity in the iron stores — plasma — intestinal-mucosa system, possibly regulated by alterations in the transferrin level (Laurell 1947, 1951, 1952). Granick (1946), on the other hand, considers the formation of ferritin in the cells of the intestinal mucosa to be of central importance in the genesis of the 'mucosal bloc'. In later papers he seems, however, to be more reserved towards this assumption and says (1951): 'whether changes in ferritin concentration are directly connected with the mucosal bloc, or whether the presence of much ferritin merely indicates that the mucosal cells are saturated with respect to some iron compounds more closely allied with the mucosal bloc, has not been determined'.

4. The plasma transport of iron

In 1925 Fontés and Thivolles called attention to the fact that a small amount of non-haemoglobin-bound iron was present in plasma. The existence of the plasma iron, which amounts to about 0.1 mg per 100 ml was confirmed and further elucidated during the next two years (Barkan, 1927; and others). Comprehensive experimental and clinical investigations on serum iron are found in particular in monographs by Thoenes & Aschaffenburg (1934), Heilmeyer & Plötner (1937), Skouge (1939), Vahlquist (1941), Büchmann (1941), Vanotti & Delachaux (1942 and 1949), Laurell (1947 and 1952), and Hemmeler (1951).

The plasma iron merits attention, mainly because of its central position in the transport of iron by the intermediate haemoglobin metabolism. The plasma iron functions mainly as a link in a closed cyclic process (haemoglobin—plasma-iron—haemoglobin) which is more or less independent of absorption, excretion, and storage. Every day 20—24 mg of iron is transported as plasma iron to the bone marrow solely for the production of haemoglobin. According to Huff *et al.* (1950), the total average amount of iron normally turned over as plasma iron has been calculated to 27 mg per day. This seems to be regulated mainly by the rate of erythropoiesis.

The combination of iron in plasma has received much attention since the discovery of the serum iron. Barkan (1927) was the first to show that the iron of serum is non-dialysable at the pH of the blood, but dialysable after acidification. He also observed that iron does not appear in an ultrafiltrate unless acidified (Barkan, 1933). It was soon confirmed that the iron in serum is protein-bound. Fractionation of sera with half-saturated ammonium sulphate precipitates the iron quantitatively with the globulins (Starkenstein & Harvalik, 1933; Barkan & Schales, 1937). Similar investigations using natural plasma containing radio-active iron indicated on the other hand that the iron here is bound to the albumin fraction (Yoshikawa *et al.*, 1942). Using electrophoresis Vahlquist (1941) found that iron in the serum wandered with both the albumins and the globulins in natural serum, but mainly with the alpha- and beta-globulins.

In 1945 Holmberg & Laurell demonstrated that serum from healthy subjects is capable of combining with added iron up to about 300 gamma per cent in firmly-bound form, which, it was suggested, was an iron-protein complex. The figures of Holmberg & Laurell agreed with the serum iron levels due to the 'braking effect' of Waldenström (1944). He and Skouge (1939) thus found that the iron concentration in serum after injections of 10 mg $Fe^{++\,+}$ as a simple salt solution never reached the expected values, but stopped at about 300 gamma per cent. This saturation limit of serum was further in-

vestigated by LAURELL (1947). He found among other things that iron added to serum above the iron-binding capacity is dialysable and can be adsorbed onto AlC_{γ}.

Ultimately it was obvious that the values at the braking point, the saturation limit, and the total iron-binding capacity of the special iron-binding protein, transferrin, are due to the same factor.

5. Transferrin, the iron-binding component in plasma

During their investigations on the saturation limit HOLMBERG & LAURELL (1945) observed that ferrous iron added to serum gives a change in serum colour from yellow or yellow-green to yellow-red. By photometry at about 5000 Å this change in serum colour was measured as a definite increase in extinction, which developed successively on repeated addition of small amounts of iron until the saturation limit was reached. Independently SCHADE & CAROLINE (1946) made the same observations. They also demonstrated fully that the change in colour was due to the formation of a special iron-protein complex. This was first shown to be concentrated in the fraction IV–3,4 by SCHADE & CAROLINE. Further subfractionation, however, led to the concentration of the iron-binding component into the new fraction IV–7 (SURGENOR *et al.*, 1949). Finally, the component was crystallized in a stable iron-saturated state by LAURELL & INGELMANN (1947), and in a mainly iron-free form by KOECHLIN (SURGENOR *et al.*, 1949).

The iron-binding protein appears in the literature under different names. The synonyms nowadays used are '$beta_1$-metal-combining globulin' (SURGENOR *et al.*, 1949), 'siderophilin' (SCHADE *et al.*, 1949), and 'transferrin' (HOLMBERG & LAURELL, 1947). Because it seems to be the best the last name will be used here.

Iron-free transferrin is colourless, whereas the iron-protein complex is salmon-coloured with a broad absorption maximum at 4700 Å. This fact has been very useful in spectrophotometric determinations of the iron-binding capacity in clinical practice (RATH & FINCH, 1949; CARTWRIGHT & WINTROBE, 1949).

Transferrin (mol. wt. about 90000) can bind 1.25 gamma of iron per milligram protein, corresponding to two atoms of iron per molecule. Normal human plasma has been estimated to contain about 2 ½ g transferrin per litre plasma, and is capable of binding approximately 3 mg of iron. Transferrin iron is always in the ferric state and ionic form, as shown by MICHAELIS (see SURGENOR *et al.*, 1949) in measurement of the magnetic susceptibility. Whether ferric or ferrous iron is added, however, the same amount is taken up in complex form.

A generally accepted opinion on the uptake of iron by plasma is that trans-

ferrin takes up free iron ions from destroyed haemoglobin, iron stores, and cells of the intestinal mucosa. More controversial views exist about the liberation of iron from plasma. One assumption is that the whole Fe-transferrin molecule leaves the blood stream (FLEXNER *et al.*, 1948). The facts available do not support this theory, as has been stressed by LAURELL (1951). According to him they suggest instead that the iron leaves the blood stream in ionized form. Transferrin is thus probably a true carrier of iron just as haemoglobin is a carrier of oxygen. The direction of the transport is probably regulated by differences in the ionic concentrations of iron, as stated below.

In healthy human subjects the transferrin is only saturated to about one third of its total iron-binding capacity (TIBC) by the serum iron (LAURELL, 1947). The saturation index, i.e. the serum iron (SI) in per cent of the TIBC, lies at about 33 per cent, and the quotient, SI/UIBC (UIBC = unsaturated iron-binding capacity), at about 0.7. Changes in these relations occur after certain patterns, particularly in iron deficiency states. The typical alterations in this quotient and in the absolute values for SI and TIBC made LAURELL to suppose that the transferrin level is of importance in the mobilization of iron and iron absorption. According to him the hypothesis can be explained by the reversible reaction $Fe^{++} + \text{Fe-free-transferrin} \rightleftarrows \text{Fe-transferrin}$ with the corresponding equilibrium equation,

$$\frac{[\text{Fe-transferrin}]}{[\text{Fe-free-transferrin}]} = K \cdot [Fe^{+++}]$$

If this opinion is accepted transferrin should serve as a vehicle for the transport of iron in plasma, and as a regulator of the mobilization of iron from the depots and of the absorption of iron from the cells of the intestinal mucosa. YUILE *et al.* (1950) have shown, however, that in iron-deficient dogs full transferrin saturation, artificially produced by injection of iron salt solutions, did not impair the absorption of radio-active iron. They concluded that the transferrin and its relative saturation played but a little role *per se* in the control of iron absorption. Their results are supported by the observations of SMITH *et al.* (1952), who found that a low and almost completely saturated total iron-binding capacity did not seem to restrain the absorption of iron in nephrotic children excreting large amounts of transferrin and its attached iron.

6. Clinical aspects of serum iron and iron-binding capacity in adults

The serum iron values in healthy adults recorded by different authors have recently been collected by HEMMELER (1951, table 15). Representative investigations on Swedish populations have been made by VAHLQUIST (1941)

TABLE I

	Method	Investigator	Year	TIBC, gamma per cent		
				Men	Women	All cases
Saturation brought about *in vivo*	Serum iron determination on the five-minutes specimen of the 10 mg intravenous tolerance test	BROCHNER-MORTENSEN	1943	361 (8)	(2)	359 (10)
		WALDENSTRÖM	1944	—	291 (18)	291 (18)
		GREENBERG *et al.*	1947			315 (13)
		TÖTTERMAN	1949	376 (13)	402 (13)	389 (26)
		BRENDSTRUP	1950	(2)	294 (9)	304 (11)
		GITLOW & BEYERS	1952	289 (10)	—	289 (10)
Saturation brought about *in vitro*	'Chemical principle' of HOLMBERG & LAURELL (1945)	HOLMBERG & LAURELL	1945	(6)	(4)	312 (10)
		LAURELL	1947	315 (61)	315 (39)	315 (100)
		DAVIES *et al.*	1952	304 (20)	320 (20)	312 (40)
	'Physical principle' of SCHADE & CAROLINE (1946)	RATH & FINCH	1949	311 (15)	288 (15)	300 (30)
		CARTWRIGHT & WINTROBE	1949	347 (15)	371 (15)	359 (30)
		SMITH *et al.*	1950			364 (11)
		VENTURA & KLOPPER	1951	—	328 (25)	328 (25)
		BRAUNSTEINER *et al.*	1952	343 (17)	352 (8)	—

The total iron-binding capacity (TIBC) in healthy adults according to different authors. Number of cases in brackets.

and LAURELL (1947) (100 subjects each). VAHLQUIST found an average of 142 (48—263) gamma per cent in males and 123 (53—210) in females. With a somewhat different method the corresponding values of LAURELL were 124 (70—214) and 108 (57—196). The sex-difference is statistically significant, according to LAURELL. This fact has been put in relation to the smaller fraction of storage iron per kg body-weight in women (LAPICQUE, 1947; ROTH *et al.*, 1951), but the essence of the mechanism is unknown.

As was first stressed by VAHLQUIST (1941) the serum iron shows pronounced diurnal variations with higher morning than evening values in healthy subjects living an ordinary life with a normal diet. This important fact has not been taken into consideration by some workers investigating the normal serum iron. The differences, however, are as large as up to 30 per cent, and must be taken into account in evaluating serum iron levels (VAHLQUIST, 1941; HØYER, 1944; WALDENSTRÖM, 1946; NILSSON, 1947; and others). The lowest values are apparently reached during the periods of greatest activity (HAMILTON *et al.*, 1950). A factor of great importance for the daily variations seems to be the duration and intensity of sleep (HEMMELER, 1944, 1951). After a short and restless night the fasting values in the morning are as low as in the evening, and a total reversal of the diurnal rhythm is found in people working at night

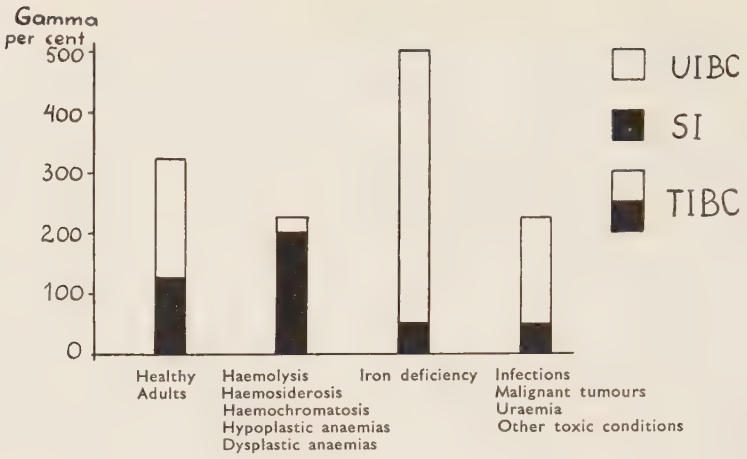

Fig. 1. *Characteristic changes in the serum iron and the iron-binding capacity in conditions with overflowing iron stores, iron deficiency, and toxic states.*

and sleeping during the day (Waldenström, 1946). Another factor influencing the diurnal variations appears to be the intake of food, as the serum iron values were found to increase during the day in fasting individuals (Jacot, 1951).

The average TIBC in healthy adults ranges from 300 to 360 gamma per cent. The figures of different authors are quoted in table I and compared with various serum iron values taken 5—15 minutes after a 10 mg intravenous iron tolerance test, i.e. the direct measurement of the TIBC by means of Waldenströms 'braking effect' (1944), a technique, however, only valid with special reservations (Hagberg, 1953a).

Whereas the serum iron level is subject to diurnal variations under normal conditions and rapid fluctuations during iron tolerance tests and in various pathological states, the TIBC appears to be much more stable (Laurell, 1947, 1952). No immediate changes have been observed after oral or intravenous iron tolerance tests, nor have diurnal variations been found (Laurell, personal communication; Smith *et al.*, 1952).

When the iron-balance is disturbed the serum iron level and the iron-binding capacity change according to certain typical patterns (Laurell, 1947, 1951, 1952; Rath & Finch, 1949; Cartwright & Wintrobe, 1949; Ventura & Klopper, 1951; Braunsteiner *et al.*, 1952). For the understanding of the general physiology of iron the following principal changes in states of iron deficiency and of iron excess are fundamental (fig. 1). Pure iron deficiency and most other conditions with increased requirements of iron (e.g. pregnancy and post-haemorrhagic states) are characterized by a low serum iron, increased UIBC and TIBC, and, consequently, a low saturation index and a low SI/

UIBC quotient. On the other hand, conditions with an increased storage of iron (haemosiderosis, haemochromatosis, haemolytic anaemias, and anaemias of dysplastic, hypoplastic, or aplastic origin) are characterized by an increased serum iron, a decreased TIBC, and a high saturation index and quotient. Finally in infections, various intoxications, and malignant tumours, the two factors vary in a specific manner independently of the iron supply; this will be further discussed below.

CHAPTER II

Iron Metabolism in Infancy and Childhood

Interest in the special problems of iron metabolism in the new-born and growing organism dates from BUNGE's early investigations on iron in young mammals (1889—1892). Among other things he and his school (ABDERHALDEN, 1898 and HÄUSSERMANN, 1897) were able to show that the amount of tissue iron in a young rabbit, dog, or cat, was high at birth, remained nearly constant during the first weeks of life, and later diminished. He held that the liver in particular of the new-born animal contained a large store of iron which could be drawn upon during the period of lactation as the milk was found to be a proportionally poor source of iron. In 1902 BUNGE pointed out the importance of his findings in infant feeding, and suggested that infants fed too long on milk would probably become anaemic after exhaustion of the liver store.

Although we now know that the magnitude and the physiological importance of the fetal liver iron stores were overemphasized in infants, and other iron sources underestimated, by these pioneer investigators, the same problems remain of interest. The rapid changes in iron balance during intrauterine and early extrauterine life always make the study of available iron stores a central factor in investigations on iron metabolism in growing organisms.

1. The storage of iron in the fetus and the new-born

The total body iron in a full-term, new-born infant amounts to about 300 mg (HUGOUNENQ, 1899; CAMERER & SÖLDNER, 1900; JERLOV, 1934; IOB & SWANSON, 1938; LINTZEL *et al.*, 1943; WIDDOWSON & SPRAY, 1951), i.e. one and a half times the amount per kg body-weight in adults. Most of the iron is acquired during the last two months of intrauterine life (JERLOV, 1934; SWANSON & IOB, 1939), as can be seen from figure 2. The curves illustrate clearly that a large quantity of iron is lost through premature birth.

During early fetal life the storage iron fraction is mainly formed by an increase in the concentration of tissue iron, later solely by a growth of the storage organs (RAMAGE *et al.*, 1933; IOB & SWANSON, 1938). As far as possible the fetus seems to furnish itself with the iron needed at the expense of the mater-

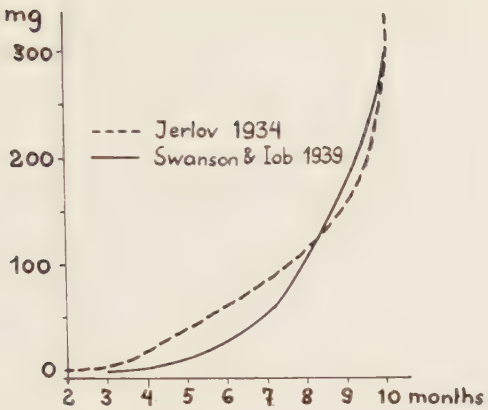

Fig. 2. *The total body iron of the human fetus according to* Jerlov (*1934*), *and* Swanson & Iob (*1939*).

nal iron stores (Jerlov, 1934). Studies with radio-iron absorption tests (Oettinger *et al.*, 1949) thus suggest that adequate iron stores are present at birth, regardless of the maternal haemoglobin level. It has further been found (Strauss, 1933) that children of mothers with a pronounced iron deficiency anaemia never present any symptoms of iron deficiency at birth though their iron stores are somewhat smaller than the average (Toverud, 1935).

In spite of an iron flow favouring the fetus, the fetal depots do not contain excessive amounts of iron at birth. Widdowson & Spray (1951) found that the liver contained no more than 30—50 mg of inorganic iron, or some 15 per cent of the total body iron, available for growth. Probably because of difficulties in exact estimation of the storage iron fraction a wide range of average values and individual variations is to be found in the literature (Gladstone, 1932; Ramage *et al.*, 1933; Toverud, 1935; Iob & Swanson, 1938; Brückmann & Zondek, 1939; Lintzel *et al.*, 1943; Roth *et al.*, 1951).

The existence of a continued post-natal iron storage during the first two months of life was first stressed by Schwartz *et al.* (1924), and Gladstone (1932). It is caused by a partial redistribution of haemoglobin iron to the depots after the adjustment of the haematopoietic system to extrauterine life following the intrauterine existence poor in oxygen. The importance of the post-natal storage of iron has been pointed out in particular by Stearns & McKinley (1937), who held that the blood constitutes the main iron store in the baby. They calculated the post-natal stores to be 250—300 mg iron. Even if these values are somewhat too high (McCance & Widdowson, 1951), they nevertheless emphasize the contrast to the 50 mg present in the liver at birth.

The development of the siderosis has been demonstrated by Langley's

(1951) recent histo-chemical investigations which indicate a temporary fall in the visible iron content of the storage organs during the first three days of life, followed by a progressive rise over the next 3—4 weeks. After 2—3 months there is a successive decrease in siderosis. These findings are roughly in agreement with chemical analyses of the iron content of the liver and spleen at various ages (Ramage *et al.*, 1933; Brückmann & Zondek, 1939; Lintzel *et al.*, 1943; Widdowson *et al.*, 1951). Thus the total amount of iron in the storage organs seems to be somewhat larger in the age group ½—2 months than in the new-born. There is then a sharp drop during the second half of the first year. The minimum average values are not reached until the age of 2 years. After that time the values are found to rise slowly again, reaching the adult level at approximately 20 years of age.

In addition to the iron stored during the fetal period, the food is the second source of iron available to the growing infant. Until recently the extent of this supply was unanimously considered to be very small, as the quantity in breast milk averages not more than about 50 gamma per cent (Lesné *et al.*, 1930; Wallgren, 1932; Feuillen & Plumier, 1952), and is about the same in cow's milk. In 1951, however, McCance & Widdowson asserted that a baby probably increases its total body iron by some 40 per cent during suckling (6 mths.), and that this iron might be obtained from the breast milk. Their findings are, on the other hand, contradicted by the results of studies with radio-iron transplacentally transferred (Smith *et al.*, 1950), which demonstrate that dietary iron makes only a negligible contribution to the haemoglobin during the six months following birth.

So far it thus seems difficult to say for certain whether blood-iron or milk-iron is the more important factor in maintaining iron-balance during the first half year of life. Be that as it may, the fundamental fact is that even a small restriction in any one of the two sources of iron mentioned probably causes iron deficiency. In other words, the margin of safety seems to be small. Symptoms of iron deficiency during the second half of the first year of life have been described 1) in untreated full term infants whose mothers had an iron deficiency anaemia during pregnancy (Strauss, 1933; Jerlov, 1934; Mackay, 1935), and 2) in infants whose umbilical cords were clamped immediately after birth, depriving the baby of considerable amounts of haemoglobin iron (Wilson *et al.*, 1941; contradictory results have, however, been published by Selander, 1945), and 3) in infants with inadequate food or nutritional disturbances (Mackay, 1931, 1946).

2. Blood morphology in relation to the changes in depot iron

The relative anoxia of intrauterine life requires an increased capacity for the transport of oxygen. The mean haemoglobin concentration of capillary blood at birth is thus as high as 19.5 gram per cent. The red cells are not increased quite as much, amounting to 5.4 million per cu.mm. (VAHLQUIST, 1948). After a temporary rise in the very first days both values gradually decrease during the following eight to ten weeks. During this period the initial macrocytosis is successively supplanted by a slight microcytosis. Hence the 'colour index' diminishes from supernormal to slightly subnormal values.

The fall in the red blood count during this first stage parallels the developing post-natal siderosis in the liver. Both have usually been attributed to excessive haemolysis following birth (for references see KÜNZER's monograph, 1951). There is, however, no convincing evidence of hyperhaemolysis (VAHLQUIST, 1941; FINDLAY *et al.*, 1947; SCHÄFER, 1949; LANGLEY, 1951), nor do survival studies using the Ashby method (MOLLISON, 1951) support an increased erythrocyte break-down of any importance in the new-born. On the other hand, the 'physiological anaemia' of the new-born and the increased content of iron in the liver might well be interpreted as being caused mainly by suppression of the erythropoietic activity of the bone marrow (JOSEPHS, 1932; VAHLQUIST, 1941; SCHÄFER, 1949) together with the post-natal falling off of extramedullary haematopoiesis (LANGLEY, 1951).

The second stage begins about three months after birth and is characterized by an unchanged haemoglobin level and a slightly rising red cell count, indicating increasing microcytosis. These changes in the blood picture corre-

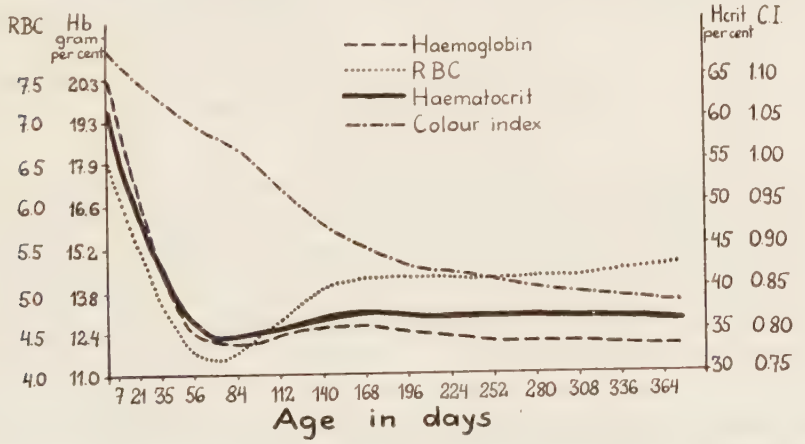

Fig. 3. *Changes in haemoglobin, red cell count (RBC), haematocrit, and colour index (C.I.), in breast-fed infants during the first year of life (according to* HORAN, *1950).*

spond to the stage of decreasing iron storage in the depots. The various alterations in the red blood picture during the first year of life are shown schematically in fig. 3, from the results of a recent investigation by HORAN (1950).

3. Serum iron in early life

The first investigation on normal serum iron values in infancy and childhood was made by THOENES & ASCHAFFENBURG (1934). In spite of a troublesome and somewhat unreliable method, their observations are still to a large extent relevant. In 1941 VAHLQUIST's important monograph was published. In a normal series of about 350 healthy infants and children he studied the various changes in serum iron from the first day of life to puberty. He also estimated the values in fetal blood at different stages of gestation, and in umbilical cord blood from full term infants at delivery. The values given below all originate from VAHLQUIST's investigations (1941 and MÖLLER & VAHLQUIST, 1946). The changes during the first year of life are given in fig. 4.

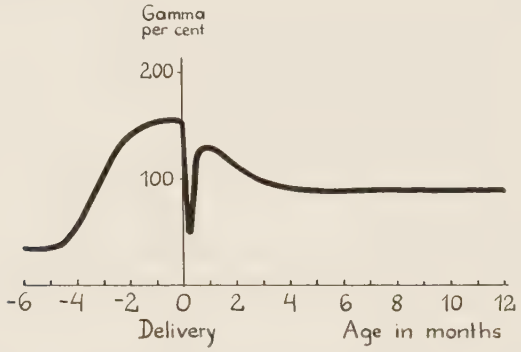

Fig. 4. *The variations in the serum iron during fetal life, at birth and during the first twelve months (after* VAHLQUIST, *1941, revised figures 1946).*

During early fetal life the serum iron level seems to be low. Until the sixth month of gestation values as low as 20—40 gamma per cent can be measured. There follows apparently a successive increase culminating in the strikingly high final level of about 140—160 gamma per cent, reached a few weeks before term. Thus the changes in serum iron during fetal life roughly parallel those of the total body iron and the stored iron.

During the first 24 hours of life there is a sharp drop in the serum iron. The values decrease from the high level of about 160 gamma per cent at birth down to some 50 gamma per cent. During the following two weeks there is again a rise to a level somewhat lower than that of the umbilical cord. The

serum iron values during the remainder of the first two months, i.e. the period of increasing storage of iron, have not been examined separately. VAHLQUIST's 24 cases in the age group 1—6 months show, however, a successive decrease to an average of some 90 gamma per cent (1946, revised values) during the second half year of life. This level is maintained until at least 2—3 years of age. At the age of seven years the mean level has risen again to about 100 gamma per cent. The female adult level is reached by both sexes at pre-puberty, whereas the decided increase in the male values apparently occurs between the ages 15—20 years.

The initial drop in the serum iron on the first day of life seems to be best explained by the cessation of the transfer of iron from the mother. The following successive rise is compatible with the beginning post-natal storage during the hyporegenerative stage of haematopoiesis.

The most debated changes refer to the second fall which takes place gradually after the first 2—3 months of age, the values then remaining stationary over at least the following two years. In connection with the microcytosis and the low content of stored iron it is tempting to assume that children in this age group have a real iron deficiency anaemia. According to VAHLQUIST (1941) the changes in the blood occurring at this time are, however, not an expression of sideropaenia but are physiological manifestations. The interpretation of the various findings and opinions will be further discussed in relation to my own investigations.

As in adults the serum iron level in children is also subject to diurnal variations. These have been studied by VAHLQUIST (1941), SCHÄFER (1949), and MAURER (1952). The same diurnal rhythm as in adults has generally been found, even in infants of less than one year of age (VAHLQUIST, 1946 — see MÖLLER & VAHLQUIST). It seems, however, to be less pronounced during the

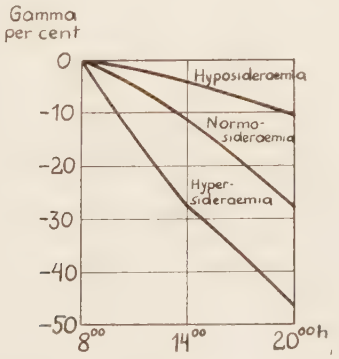

Fig. 5. *Average diurnal lowering of the serum iron in 48 children aged 3—13 years (from* MAURER, *1952).*

first three months of life (VAHLQUIST, personal communication). The degree of the fluctuations seems to be related to the initial fasting level (MAURER, 1952, fig. 5).

4. Earlier investigations on the iron-binding capacity in infancy and childhood

In spite of the interesting fluctuations of the iron balance taking place during the first years of life, the corresponding changes in the TIBC have not yet been satisfactorily established. When this work was started in 1950 only the values in the new-born infant at parturition were known (LAURELL, 1947). To quote LAURELL: 'The mean value for 25 new-born children was 226 $\pm$ 10 gamma per cent, with a serum iron concentration of 147 gamma per cent. How rapidly the normal adult value for iron-binding protein is attained is still to be investigated.'

In 1950 SMITH and associates determined the iron-binding capacity in children with Mediterranean anaemia, and also, as a comparison, in 11 healthy children varying in age between 2 weeks and 10 years. Recently SMITH *et al.* (1952) published their investigations on the serum iron and the iron-binding capacity in healthy infants and children and in young patients with various blood disorders. Their normal series consists of 51 subjects, of whom 22 were less than one year of age. Only 7 determinations, however, were made in the important stage 1—6 months, where the available storage iron is altered more than at any time in later life.

The results obtained in the investigation carried out by SMITH and associates will be discussed in relation to my own investigations.

CHAPTER III

Methods

1. Determination of the serum iron

The determinations of the serum iron were made according to the well-established principles first set out by HEILMEYER & PLÖTNER (1937). A micro-modification of VAHLQUIST's method (1941) was used.

The principle of the method is as follows. The protein-bound iron of the serum is liberated after acidification with hydrochloric acid. The proteins are fully precipitated with trichloracetic acid. The protein-free supernatant iron salt solution is adjusted to a suitable pH and the ferric iron reduced with hydroquinone to ferrous iron, which is converted into a coloured iron complex by the addition of ortho-phenanthroline hydrochloride. The intensity of the colour, read spectrophotometrically, is proportional to the iron concentration.

Technique

Cleaning of glassware. All glassware used was treated with potassium dichromate in concentrated sulphuric acid and washed in repeated changes of iron-free water (passed through a cation exchange column).

Reagents and solutions.

6 M hydrochloric acid (pro analysim)
20 % trichloracetic acid (pro analysim)
Concentrated ammonia (pro analysim)
M/2 hydrochloric acid
para-nitrophenol, 1 %
Hydroquinone, 2 %
O-phenanthroline hydrochloride, 1 %

Analysis. To 0.3 ml serum are added 0.3 ml water (iron-free) and 0.3 ml 6 M hydrochloric acid, with constant shaking. After 15 minutes 0.6 ml trichloracetic acid is added, again with shaking. 20 minutes later the mixture is centrifuged for 15 minutes (at 1 100 *g*, Corda). The supernatant is cautiously taken off with the aid of a Pasteur pipette and 1 ml used for the analysis. After the addition of one drop of p-nitrophenol as indicator the mixture is neutralized with concentrated ammonia to the exact point when a yellow colour is first seen. The mixture is then acidified with M/2 hydrochloric acid, 0.03 ml more than necessary to make the colour disappear again. One drop of hydroquinone and one drop of o-phenanthroline hydrochloride are added, and the final volume calculated according to VAHLQUIST. After half an hour or more the extinction value is read against water in a Pulfrich photometer with 50 mm microcuvettes and filter S 50.

Blanks were obtained by using iron-free water instead of serum. They ranged from 14 to 35 gamma per cent, average 24, the variations depending mainly on the purity of the trichloracetic acid used.

The formula for converting the values to gamma per cent can be found in VAHLQUIST's monograph (pp. 73—74).

Error of measurement

On the basis of 100 arbitrarily chosen duplicate determinations the standard error was calculated to $\sigma_m = 6$.[1] This implies that practically no single estimation deviates more than 18 gamma per cent from the real value.

All values recorded in this investigation are the mean of duplicate determinations for greater accuracy, σ_m being $6/\sqrt{2}$ or 4.2. Thus the error of the measurement applied here only amounts to about 4 per cent when the serum iron is 100 gamma per cent.

Comparison with the original method of VAHLQUIST

30 samples were simultaneously analysed by VAHLQUIST's original method and the micro-modification. Each value used for comparison was the mean of duplicate determinations. The figures corresponded well, the correlation coefficient being $+0.98 \pm 0.01$ for samples with serum iron levels between 72 and 175 gamma per cent. In 8 cases identical values were found. The average deviation between the results was 4 gamma per cent and was not systematic. With VAHLQUIST's method the mean value of the group was 113.53 gamma per cent, and with the micro-modification 113.66. As can be seen, the difference is insignificant (0.13 ± 1.1).

Discussion

The method used differs from VAHLQUIST's in the following ways.

1. 0.3 ml serum was used instead of 2 (1) ml.
2. Before acidification the serum was diluted with its own volume of iron-free water.
3. After precipitation of the proteins the sample was centrifuged instead of filtered.

The amount of serum was reduced, partly as a link in the attempt to obtain micro-methods for laboratory analyses in paediatric practice, and partly

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[1] The formula used was $\sigma_m = \sqrt{\frac{\Sigma d^2}{2n}}$ (after DAHLBERG, 1940), where d is the difference between the two values obtained for the same sample, n the number of differences, and σ_m the error.

to try to cut down the size of the blood sample needed for determination of the TIBC. The volume of 0.3 ml serum for the analyses was chosen after preliminary tests with diminishing amounts of serum. It was found to be the least amount which could be safely employed.

With the small amounts of serum used dilution to a larger volume was necessary to bring the final amount to slightly more than 1 ml for spectrophotometric determinations in the microcuvettes of the Pulfrich Stufenphotometer. The disadvantage of the dilution was that the errors of the readings were doubled. On the other hand far fewer opalescent reaction-mixtures following the addition of hydrochloric and trichloracetic acids were noticed.

Centrifuging was also adopted to make a larger amount of reaction-mixture available for the final reading, as quite a lot of the solution is lost during filtration. No inconvenience was suffered from this alteration of the method. On the contrary, one source of error, the possibility of accidentally introducing iron with the filter-paper, was eliminated.

2. Determination of the total iron-binding capacity of serum

At present it is possible to determine the TIBC in three quite different ways. The oldest, originally used by SKOUGE (1939) and WALDENSTRÖM (1944), is to examine the 'five minute specimen of the ten milligram intravenous iron tolerance test'. It has recently been revived by CARTWRIGHT & WINTROBE (1949) and GITLOW & BEYERS (1952), who consider it to be the simplest and most satisfactory way to measure the TIBC. The method of HOLMBERG & LAURELL (1945) and LAURELL (1947) is based on the fact that iron added to serum in excess of the TIBC reacts with $\alpha\alpha_1$-dipyridyl or ortho-phenanthroline in the presence of a small amount of sodium hydrosulphite. The third method is based on the investigations of SCHADE & CAROLINE (1946). They found that progressive development of a salmon-red colour occurs on the addition of ferrous iron to $beta_1$-globulin until the protein becomes saturated. At the point of saturation there is a sharp break in the development of the colour (fig. 6). From the amount of iron needed to reach this point the UIBC may be calculated (RATH & FINCH, 1949; CARTWRIGHT & WINTROBE, 1949). The TIBC is then obtained by adding the figures of the UIBC and the serum iron.

The original intention was to employ the method of LAURELL (1947). The advantages are that 1) it seems to be the most reliable method especially when the UIBC is low; 2) it can be used on any kind of serum without limitation; and 3) it may be carried out on less than 1 ml serum (0.2 ml according to DAVIES *et al.*, 1952), which is of importance when dealing with children.

When this work was started it was, however, impossible to obtain fresh

sodium hydrosulphite *pro analysim*. As intravenous iron tolerance tests in children are unsuitable and unpractical and this method commonly gives unreliable values unless the test dose is closely related to the body-weight (HAGBERG, 1953a), the third method, based on the principles of SCHADE & CAROLINE, was adopted. The modification by RATH & FINCH (1949) was used, as the same type of spectrophotometer was available.

Technique

Solutions required. Standard iron solution containing 20 gamma of ferrous iron per ml, prepared by diluting 14 mg of ferrous ammonium sulphate + 0.5 ml 1 M acetic acid in 100 ml iron-free water.

Physiological saline (iron-free).

Analysis. Serum from fasting subjects was used (see p. 38). The analyses were performed in a Coleman spectrophotometer, Model 14, with square cuvettes (14—307) 1.3 cm deep.

2 ml of serum and 3 ml of saline are pipetted into one of the cuvettes, and 5 ml saline is placed in the other. The transmission for the last cuvette is corrected to 100 per cent and thus serves as a blank. Addition of iron to this cuvette was found to be unnecessary. After 10 minutes the transmission of the serum dilution is measured several times and used as the basic value. Standard iron solution is then added to the sample in quantities of 0.05 ml at a time, and the cuvette rocked to mix thoroughly. Several readings are taken 2 minutes or more after each addition of iron. Before each reading the blank is checked. Fractionated addition of iron is thus made until no further change in the transmission occurs on three successive readings. The difference in transmission is converted into a percentage of the basic value. The figures plotted on graph paper give two straight lines, one representing the decrease in transmission, and the other the constant transmission of the end-point (fig. 6). The point of intersection of these two lines represents the amount of iron added, i.e. the UIBC. The TIBC is obtained by adding the serum iron value.

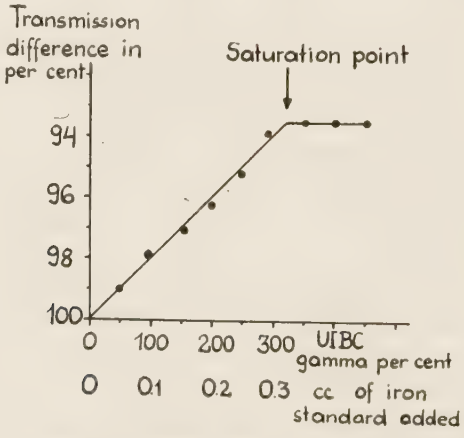

Fig. 6. *A typical iron titration curve.*

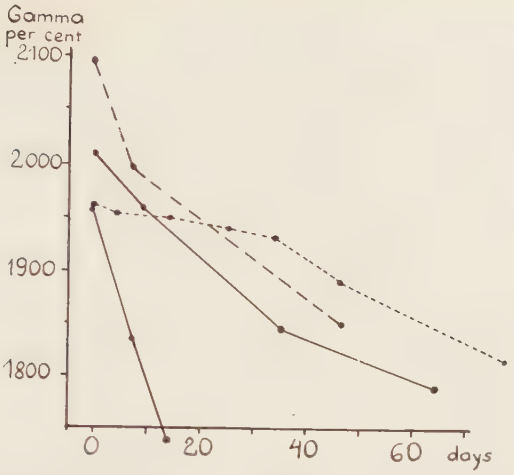

Fig. 7. *The fall of the iron concentration in the standard solution.*

Stability of the standard iron solution

The stability of the solution was checked by repeated determinations of the iron concentration. It was found that this steadily fell, the rate, however, varying greatly from solution to solution. Some examples are given in fig. 7.

The cause of the fall was found to be a deposition of iron on the walls of the vessels. This source of error is of no practical importance when the UIBC is low (50—100 gamma per cent), but when it is raised the error may be considerable. Throughout this investigation, therefore, the fall in the iron concentration of the standard solution has been taken into account.

Error of measurement

The error was calculated[1] from duplicate determinations made on 148 samples representing different levels of the UIBC. The average standard error was found to be 13 (table II), which corresponds to about 5—10 per cent of the values when the UIBC exceeds 100 gamma per cent. As the absolute error is practically constant at all levels of the UIBC the method is far less reliable for levels between 50 and 100 gamma per cent. Values below 50 gamma per cent cannot be determined by the method of Rath & Finch but may be roughly calculated from the standard curve described below (fig. 8). As the standard iron solution used contains minimal amounts of ferric iron there must be, in addition to the above-mentioned error of the method, a systematic error which, however, is probably inconsiderable. It is due to the fact that

[1] According to the formula $\sigma_m = \sqrt{\frac{\Sigma d^2}{2n}}$ (see footnote p. 26).

TABLE II

UIBC level	No. of blood samples	S. D.	S. D. in per cent of mean UIBC
< 100	19	13	21
100—200	40	14	9
200—300	62	11	5
300—500	27	16	4
All values	148	13	

Determination of the error of the method by duplicate estimations at different levels of the unsaturated iron-binding capacity (UIBC). S. D. = standard deviation.

the transferrin combines at a slower rate with trivalent than divalent iron (SURGENOR *et al.*, 1949), and consequently also takes a longer time to develop the corresponding increase in serum colour.[1]

Checking the method by means of serum iron determinations following iron tolerance tests

Intravenous iron tolerance tests. The total iron-binding capacity (SI + UIBC = TIBC) was initially measured and then compared with the serum iron level of specimens collected 5, 15, and 60 minutes after intravenous iron tolerance tests (SI = TIBC) with an ionized iron solution. Thus two of the principal methods of determining the TIBC were compared.

Apparently healthy student nurses volunteered. The iron was administered in the form of a 4 per cent solution of ferrous sulphate[2] in doses of 0.130—0.145 mg Fe^{++} per kg body-weight. The injections were made slowly over a period of 5—10 minutes. All of the women reacted with flushing, some of them also with coughing or sneezing, 'pins and needles', and slight tachycardia.

Table III demonstrates that the values for the TIBC measured before the injection of iron corresponded in every case with the serum iron levels obtained 5, 15, and 60, minutes after the injection, provided that saturation was complete. In samples where this was not so, i.e. where a residual UIBC could be shown, the TIBC determined before tallied with the TIBC measured after the injection.

Oral tolerance tests. A more physiological way of trying to reach the satura-

[1] WOLFF (Biochem. Ztschr. *322*: 344, 1952), however, has recently found that *both* ferrous and ferric iron solutions when added to serum *in vitro* give an immediate and full development of the Fe-transferrin colour.

[2] made available through the courtesy of AB Pharmacia Ltd., Uppsala.

TABLE III

No.	mg Fe per kg b.-wt.	Before injection			After injection								
					5 min.			15 min.			60 min.		
		SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC
1	0.144	47	422	*469*	369	83	*452*	333	104	*437*	321	125	*446*
2	0.143	80	215	*295*	334	0	*334*	332	0	*332*	324	0	*324*
3	0.142	139	193	*332*	359	0	*359*	333	0	*333*	324	0	*324*
4	0.142	184	118	*302*	351	0	*351*	324	0	*324*	317	0	*317*
5	0.140	110	216	*326*	330	0	*330*	329	0	*329*	297	0	*297*
6	0.140	59	223	*282*	302	0	*302*	285	0	*285*	282	0	*282*
7	0.139	129	229	*358*	310	0	*310*	343	0	*343*	333	0	*333*
8	0.138	145	164	*309*	252	69	*321*	254	61	*315*	225	86	*311*
9	0.138	59	437	*496*	527	0	*527*	525	0	*525*	467	0	*467*
10	0.135	76	257	*333*	333	0	*333*	334	0	*334*	333	25	*358*
11	0.133	88	246	*334*	324	0	*324*	315	0	*315*	306	0	*306*
12	0.133	133	198	*331*	366	0	*366*	357	0	*357*	351	0	*351*
13	0.131	44	262	*306*	—	—	—	315	0	*315*	276	42	*318*

Determination of the serum iron (SI) and the unsaturated iron-binding capacity (UIBC) before and after intravenous iron tolerance tests using injectable ferrous sulphate.

TABLE IV

Age	Sex	mg Fe given	Before iron intake			2 hours after			4 hours after			6 hours after		
			SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC
23	♀	280	68	340	*408*	224	194	*418*	432	0	*432*	—	—	—
21	♀	560	108	312	*420*	330	129	*459*	490	0	*490*	498	0	*498*
21	♀	560	88	374	*462*	390	45	*435*	442	0	*442*	430	0	*430*
25	♀	560	126	211	*337*	326	0	*326*	348	0	*348*	354	0	*354*
26	♀	560	117	252	*369*	—	—	—	388	0	*388*	406	0	*406*
28	♂	560	122	225	*347*	304	90	*394*	408	0	*408*	370	0	*370*

Oral iron tolerance tests. The iron tablets used were 'Novofer' (Pharmacia) containing 0.25 g ferrous tartrate and 0.0025 g cupric sulphate.

tion limit is to supply iron by mouth. Owing to the regulation mechanism for the absorption of iron the undamaged intestinal mucosa only permits a small amount of iron to pass into the blood, and thus the saturation limit is never reached in many healthy subjects in spite of large test-doses of iron. As iron salts are better absorbed in iron deficiency states, some of the tolerance tests were carried out on women with slight hypochromic anaemia. Iron was given in the form of 5—15 Novofer tablets (Pharmacia) containing 0.25 g ferrous tartrate (= 56 mg Fe) and 0.0025 g cupric sulphate. Full saturation was only

achieved in six cases out of seventeen. These six cases have been recorded in table IV. Two of them (nos. 1 and 2) developed nausea, blanching, and faintness. The relationship of these symptoms to a possible exceeding of the TIBC has been discussed in another paper (HAGBERG, 1953a).

It is obvious that the figures here do not tally so well as in the intravenous tolerance tests. At four and six hours after ingestion the serum iron shows a tendency to slightly higher levels than the TIBC values before the beginning of the test.

Discussion. Intravenous iron tolerance tests give the 'braking point' only when certain precautions as to the dose and the rate of injection are observed (HAGBERG, 1953a). This is due to the fact that the saturation limit for the complex-binding of iron only seems to be relative to the presence in plasma of further, and then apparently loosely-bound, iron (HOLMBERG & LAURELL, 1945; HAGBERG, 1953a). That the tests performed here really gave the 'braking point' is supported by the consistent agreement of the serum iron levels at full saturation on three separate occasions during the first hour after injection. Thus it can also be concluded from the results obtained that the determinations of the TIBC by the method of RATH & FINCH on *normal* sera gave the level of the braking point, i.e. the total iron-binding capacity (TIBC). The oral iron tolerance tests, however, do not agree quite so well, the cause of which is hard to interpret, but they point in the same direction. A simultaneous comparison with LAURELL's method would have been interesting, but for reasons mentioned previously it has not been possible to do this.

Analysis of the relationship between the magnitude of the unsaturated iron-binding capacity and the deepening of serum colour on saturation *in vitro*

LAURELL (1947) investigated whether the change in serum colour after addition of iron was directly proportional to the UIBC level calculated by his method. He found that a single estimation of the change in serum colour after iron enrichment gave an approximate expression for the UIBC. Low and normal values were, however, found to be very uncertain. He presumed that this finding was due to interfering colour reactions between his iron-ascorbic-acid solution and substances in serum other than the Fe-transferrin.

As many iron titration curves for determining the UIBC have been done during my investigations it was considered to be of both theoretical and practical interest to analyse statistically the deepening of serum colour in relation to the UIBC values measured during step-by-step saturation. Much time would be spared if one single measurement after supersaturation with iron would suffice for routine clinical use.

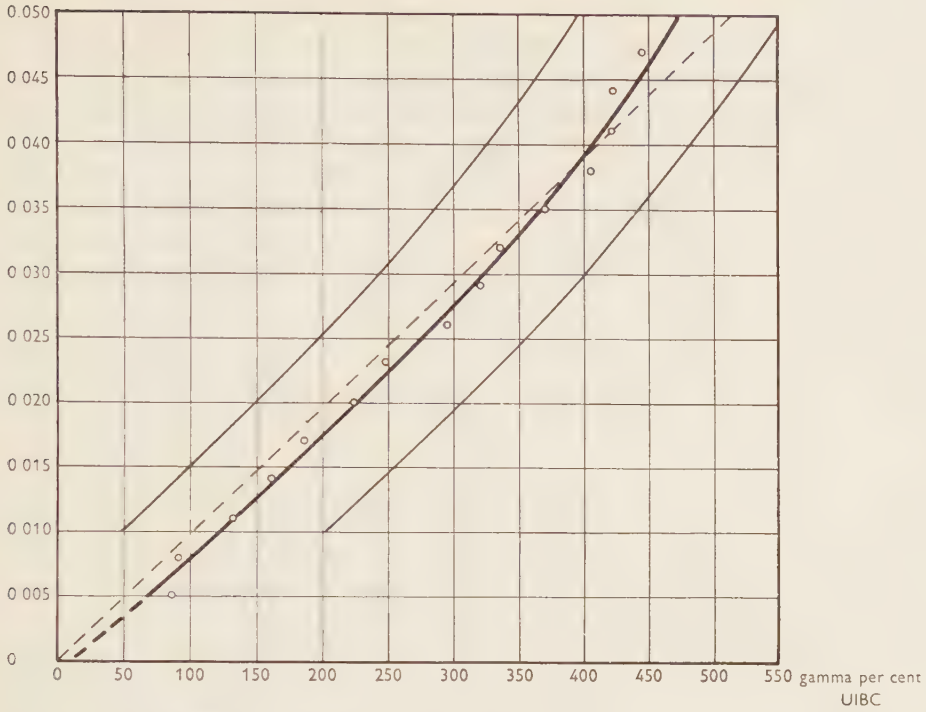

Fig. 8. *The unsaturated iron-binding capacity (UIBC) calculated from the total increase in extinction on titration with iron.* (Continuous thick line = expected mean UIBC for a given increase in extinction. Continuous thin lines = the random variations of the expected mean. Dashed line = Hypothetical relationship between the UIBC and the increase in extinction. This line has been calculated from the average increase in extinction (0.00486) of single median titration steps (UIBC = 50 gamma per cent) and the analysis refers to 177 titration curves).

The total increase in extinction at full saturation and the corresponding UIBC were evaluated in 229 sera (the increase in extinction ranged from 0.004 to 0.048 and the UIBC from 50 to 480 gamma per cent). The values were collected into 15 classes according to the increase in extinction. The class breadth was 0.003. In each class the mean UIBC was calculated, and these mean values are marked as circles in the diagram (fig. 8).

It is obvious that the first twelve mean values fall on a straight line. A linear smoothing of the mean values within the extinction region 0.008—0.035 (by the method of least squares) gives the equation $y = 15 + 10272\ x$[1]. The regression coefficient, 10272, denotes the increase in extinction when the UIBC is increased by one unit. The curve has an *intersept* equal to 15 gamma. This would imply that an average iron enrichment corresponding to 15 gamma per 100 ml is usually necessary to obtain any increase in extinction at all. This finding is supported by practical experience during analysis. Thus it was sometimes found that the first change in serum colour was remarkably small and in a few cases quite failed to appear.

[1] The constructed dashed line (fig. 8) represents the equation $y = 10288\ x$.

TABLE V

Increase in extinction value	Mean value of UIBC gamma per cent	Variations of UIBC (Probability = 0.99)
0.004	54	?
.006	77	?
.008	100	?
.010	123	44—202
.012	145	66—224
.014	166	87—245
.016	187	108—266
.018	208	129—287
.020	228	149—307
.022	247	168—326
.024	267	188—346
.026	285	206—364
.028	304	225—383
.030	322	243—401
.032	339	260—418
.034	356	277—435
.036	372	293—451
.038	388	309—467
.040	404	325—483
.042	419	340—498
.044	434	355—513
.046	448	369—527
.048	461	382—540
.050	475	396—554

Figures referring to fig. 8.

When all 15 mean values are taken into account a parabola may represent the equation[1] slightly better than a straight line. Fig. 8 shows the curve drawn with a continuous, thick line. It expresses the expected mean UIBC for a given increase in extinction. The continuous, thin lines signify the random variations of the UIBC within which in 99 per cent the actual value is to be found. The figures obtained are recorded in table V. High extinction values seemed to give a relatively smaller increase in UIBC. The curve was, however, found to be approximately linear up to extinction values of 0.035—0.040. The surprising deviation above these levels is difficult to explain.

It may be concluded that the 'one stage measurement' of the total extinction increase after enrichment of serum with a supersaturating amount of iron gives far less accurate values for the UIBC than the titration method used in this investigation. The accuracy of the former seems theoretically to

[1] $y = 5.8 + 12.25\,x - 0.0575\,x^2 \pm 31\,y$ = expected UIBC at ext. $1000\,x$ (method of least squares).

be less than 1/3 of the latter, as step-by-step addition of iron seems to keep the variations within 50 gamma per cent. In practice the differences are probably somewhat less. The 'one stage method' is, however, unreliable and gives only an approximate idea of the true UIBC.

The great variation in the increase in extinction, due to full saturation of various sera at a given UIBC level, supports LAURELL's hypothesis of interfering substances. Moreover, during the first stage of iron titration the increase in serum colour was less than expected. The extent of the change in colour which failed to appear corresponded to that calculated for the addition of about 15 gamma of iron per 100 ml. This finding further stresses that substances interfering with the expected development of the salmon-red Fe-transferrin colour are probably present in normal serum.

Substances in serum interfering with the method

The factor most affecting the accuracy of the method is obviously the presence of certain interfering substances which change the serum colour or make the serum opalescent. What they are is not known, nor is the extent of their role understood (note, however, the observations made above). RATH & FINCH (1949) and CARTWRIGHT & WINTROBE (1949) state that icteric and lipaemic sera cannot be analysed. SMITH *et al.*, on the other hand, found that bilirubin concentrations of 1.0 mg per cent did not interfere with the determinations, and concluded that bilirubinaemia of even high degree had a negligible effect. They did not, however, perform quantitative measurements in different degrees of bilirubinaemia.

As the clinical material reported below was originally intended to include new-born infants in the period of physiological icterus, it was necessary to investigate the quantitative influence of bilirubinaemia. Pure bilirubin (HOMBURGER) was added to serum from a healthy man and a series of serum dilutions of varying bilirubin concentrations made (table VI). The results showed that bilirubinaemia of about 3—4 mg per cent and above interfered with the measurements and gave lowered values. As the bilirubinaemia of week-old babies amounts to 7—8 mg per cent on an average, infants in the first weeks of life were not included in the final series. Older infants with signs of icterus were also excluded. It must be admitted at the same time that serum from the umbilical cord is usually difficult to analyse in spite of bilirubin concentrations below the critical values (1.75 ± 0.11 mg per cent according to VAHLQUIST 1941).

Corresponding investigations were also made on the interfering effect of haemoglobin in serum. It was found (table VII) that even obvious haemolysis did not significantly interfere with the results of the titration method used.

TABLE VI

Bilirubin mg per cent	Degree of visible icterus	UIBC measured gamma per cent	Transmission of light (per cent)
0.6	0	240	73.9
1.1	0	250	69.0
1.6	0	225	66.9
2.1	(+)	230	64.1
2.6	(+)	250	62.0
3.6	+	225	57.9
4.6	+	200	53.5
6.6	+ +	175	48.0
8.6	+ + +	200?	43.0
9.9	+ + + +	125	34.0

The interfering effect of various concentrations of bilirubin on the spectrophotometric measurement of the unsaturated iron-binding capacity (UIBC).

TABLE VII

Haemoglobin added mg per cent	Degree of visible haemolysis	UIBC measured gamma per cent	Transmission of light (per cent)
0.0	0	250	78.0
2.5	0	250	77.7
5.0	0	250	76.1
10	(+)	235	75.2
20	(+)	250	73.0
40	+	250	68.5
60	+	250	64.8
80	+ +	250	60.6
100	+ + +	230	56.9
200	+ + + +	185	39.5

The interfering effect of various degrees of haemolysis on the spectrophotometric measurement of the unsaturated iron-binding capacity (UIBC).

Thus the special precaution of RATH & FINCH, who consistently used syringes coated with mineral oil, could be discarded.

During the investigations it was empirically found that non-fasting sera as well as those from patients with a severely disturbed plasma protein pattern (e.g. a case of disseminated lupus erythematosus) were usually unsuitable owing to turbidity. Other cases with signs of a continuously or temporarily dysfunctioning liver may also have sera which give unsatisfactory curves. Such observations were for instance made in patients with liver cirrhoses, ulcerative colitis, and infectious mononucleosis.

Critical evaluation of the method

Summing up it may be said that this widely used method is far from perfect, but is accurate enough for measurements on normal, clear, fasting sera. It is also valid in various disease states where the serum is not discoloured or lipoid-rich, and where there is no severe disturbance of the plasma protein pattern. These limitations, however, diminish considerably the usefulness of the method, and make it inapplicable in many pathological conditions with changes in the TIBC. Thus the method may falsely suggest full saturation in, for instance, acute hepatitis, where the UIBC is in fact normal or high in spite of an often elevated serum iron level. Haemolytic anaemias and uraemia are other conditions where the method sometimes fails.

It is difficult to assess the accuracy of the method for calculating the TIBC in suitable sera. Presuming that the TIBC is constant during the day and fluctuates only insignificantly in healthy adults from day to day and month to month, the differences between comparable values from the same subject but seldom exceed 50 gamma per cent, and are usually decidedly lower, an opinion supported by the results of the iron tolerance tests.

Estimations on smaller amounts of serum

In order to cut down the amount of serum required for the determination of the TIBC, the reliability of using 1 ml (diluted 1 : 5 instead of 2 : 5) was investigated on 53 samples. Thus analyses were performed both with 2 and 1 ml of the same serum. The results were assessed statistically and found to agree well, the correlation coefficient being + 0.986 $\pm$ 0.004 (range of UIBC = 50—550). There was no systematic deviation between pairs of values. On an average the results of the determinations using 2 ml serum were 4 $\pm$ 3 gamma per cent higher, but this difference is within the limits of random variation. The standard deviation of the differences was found to be 20 gamma per cent.

In spite of this satisfactory agreement the individual 1 ml curves were found to be definitely less reliable as the deviations measured on the spectrophotometer usually became very small. Thus the use of 2 ml was found to be desirable, at least when establishing a normal series, although routine clinical determinations may be performed on 1 ml of serum.

Comparison between venous and capillary blood

Vahlquist (1941) has shown that the serum iron levels in venous and capillary blood tally well. As the values presented in this investigation refer to both venous and capillary blood samples, simultaneous analyses of the UIBC on both sorts of blood have been made in 11 cases. The results are recorded in table VIII. They confirm Vahlquist's findings on serum iron, and demonstrate that the same good agreement also applies to the UIBC measurements.

TABLE VIII

Sex Age yrs.	SI gamma per cent		UIBC gamma per cent		TIBC gamma per cent	
	venous	capillary	venous	capillary	venous	capillary
♂ 1 $^{6}/_{12}$	90 (2)	84 (2)	350 (2)	338 (2)	440	422
♂ $^{4}/_{12}$	126 (2)	128 (2)	190 (2)	188 (2)	316	316
♂ $^{8}/_{12}$	40 (2)	38 (2)	515 (2)	520 (2)	555	558
♂ 28	150 (2)	140 (2)	265 (5)	250 (3)	415	390
♀ $^{2}/_{12}$	156 (2)	148 (2)	70	75	226	223
♂ $^{7}/_{12}$	—	—	250	244 (2)	—	—
♀ $^{3}/_{12}$	—	—	250	250	—	—
♂ $^{6}/_{12}$	42	36 (2)	280	280	322	316
♂ 7	—	—	185	185	—	—
♂ $^{9}/_{12}$	92	92 (2)	370	341	462	433
♂ $^{5}/_{12}$	39[1](2)	34 (2)	300[1](2)	315 (2)	339[1]	349

[1] = arterial

The serum iron (SI) and the unsaturated iron-binding capacity (UIBC) determined on both venous and capillary blood samples taken simultaneously from the same individual. Number of analyses in brackets.

3. Collection and treatment of the blood samples

About 3—4 ml serum is usually needed to perform the determinations. If, however, the UIBC is determined on 1 ml, and single determinations of the serum iron are performed, only 1½—2 ml serum is required. Usually about 10 ml blood was collected for these investigations as duplicate determinations were always made when analysing the serum iron level, and in many cases also when estimating the TIBC. It is often difficult to obtain such large quantities of blood from infants without haemolysis occurring. The following technique was used.

All-glass syringes and chrome nickel needles ('2R2', made in France, manufactured in Sweden by AB Vitrum, Stockholm) were used for the venipunctures, which were performed in a cubital vein when possible, otherwise in the femoral vein just distal to the inguinal ligament.

Capillary blood from the heel was used in infants under one year of age. To obtain such large amounts of capillary blood as 10 ml it is of utmost importance to follow certain precautions. The following procedure was found to be satisfactory.

1. The foot is thoroughly warmed in water of 38—39°C for five minutes, dried, and the heel cleansed with ether.
2. With sharp, newly polished stilettes two moderate punctures are rapidly made close together in the heel.
3. The blood is allowed to drip into an iron-free tube, but not to run down its walls. It is helpful to have the leg hanging down. No other stasis is necessary.
4. Clotting is prevented by frequently wiping the wounds with sterile gauze.
5. Note that the blood must not be squeezed out, as this definitely favours clotting and haemolysis.

Table IX

Time in deep-freezer	UIBC measured gamma per cent
4 days	144
10 "	144
19 "	125
2 mths	120
3 "	109
4 "	143
8 "	120
19 "	150

Continuous determinations of the unsaturated iron-binding capacity (UIBC) of a serum kept in a deep-freezer for 19 months.

The samples were left for at least one hour for the clots to retract. They were then centrifuged twice. The serum was pipetted off and frozen down in a deep freezer for analysis days or weeks later.

Preserved in a frozen state at —15 to —20°C, the UIBC seemed to remain unchanged for a long time. An example is given in table IX.

All samples from the clinical material presented in this investigation were treated as above, and the analyses mostly performed within the next two or three weeks.

4. Other haematological methods employed

Haemoglobin was measured with Autenrieth-Königsbergers haemometer (standardized according to Enghoff), or Haemotest (Vedbaek) equilibrated with the former.

Erythrocytes were counted according to the usual routine clinical method (Ellermann).

Erythrocyte sedimentation rate. The micro-method described by Ström (1933) was used.

CHAPTER IV

Clinical Investigations on the Iron-binding Capacity

1. Healthy adults

The serum iron and the total iron-binding capacity (TIBC) were determined in 54 healthy adults in order to obtain a series of basic values for judging the results obtained in infants and children.

Material. The series consisted of 26 men and 28 women, most of them doctors, medical students, nurses, or student nurses. All were in good health without any symptoms of infection during the preceding weeks. The average age of the men was 29 (21—61) and of the women 26½ (21—46). The blood samples were collected in the fasting state, between 8 and 9 o'clock in the morning.

Results and conclusions. The results are recorded in table X, and are compared with those of other investigators in table XI.

The serum iron values obtained agree satisfactorily with the findings of other authors, especially with those of VAHLQUIST (1941) who used practically the same technique in his determinations. His corresponding figures were 142 ± 6.1 gamma per cent in 50 men and 123 ± 4.5 gamma per cent in 50 women. The sex-difference was not statistically significant in the present series but the same tendency was found as in larger series.

The values for the TIBC also tally well with the results of other investigators, as can be seen from table XI. The saturation index was, however, some-

TABLE X

	No. of cases	SI gamma per cent		TIBC gamma per cent		SI/TIBC per cent		SI/UIBC	
		M ± ε (M)[1]	S. D.[1]	M ± ε (M)	S. D.	M ± ε (M)	S. D.	M ± ε (M)[1]	S. D.
Men	26	137 ± 6.3	32	321 ± 7.7	39	44 ± 2.6	13	1.0 ± 0.2	0.8
Women	28	123 ± 8.3	44	338 ± 5.9	31	37 ± 2.6	14	0.7 ± 0.1	0.6
Both sexes ..	54	130 ± 5.2	38	330 ± 4.9	36	40 ± 1.9	14	0.9 ± 0.1	0.7

[1] M ± ε (M) denotes mean ± standard error of the mean. S. D. signifies the standard deviation.

The serum iron (SI) and total iron-binding capacity (TIBC) in healthy adults (fasting values).

Table XI

Investigator	Year	No. of cases		SI gamma per cent			TIBC gamma per cent				SI/TIBC per cent
		Men	Women	Men	Women	All cases	Men	Women	All cases		
									M ± ε (M)	S. D.	
Laurell	1947	61	39	124	108	117	315	315	*315 ± 3.3*	33	38
Rath & Finch	1949	15	15	106	94	100	311	288	*300 ± 9.4*	51	34
Cartwright & Wintrobe	1949	15	15	127	123	125	347	371	*359 ± 5.6*	31	35
Smith *et al.*	1950	11		—	—	163	11		*364 ± 15.4*	51	45
Ventura & Klopper	1951	—	25	—	111	—	—	328	*328 ± 9.8*	49	34
Davies *et al.*	1952	20	20	126	104	115	304	320	*312 ± 7.5*	47	37
Hagberg	1953	26	28	137	123	130	321	338	*330 ± 4.9*	36	40

The serum iron (SI) and the total iron-binding capacity (TIBC) in healthy adults according to various authors.

what higher, possibly indicating that the mean TIBC obtained might have been somewhat lower if the serum iron technique of these other investigators had been used. A small sex-difference in the TIBC, saturation index, and quotient, was found, but it was not statistically significant.

Summing up, the figures obtained in healthy adults agree with the results of other observers, and thus afford a good basis for evaluating the values obtained in infants and children.

2. Mother and child at delivery

The haemoglobin and the red cell count diminish successively during pregnancy. At the same time there is an increase in the plasma volume amounting to 25—50 per cent (e.g. Caton *et al.*, 1949; Bucht, 1951). In spite of the falling blood values there is, however, an increase in the red cell mass (Caton *et al.*, 1951; Lund, 1951) which corresponds to haemoglobin containing about 500 mg of iron (Rath *et al.*, 1949). As the average loss to the fetus is 300 mg and more iron is needed for building up the placenta and the growing uterus, the extra demand of iron during the whole pregnancy seems to be at least 1000 mg. Although most of the lowering of the blood values is probably explained by 'dilution', it is no wonder that, in addition, signs of iron deficiency may be and have been found during late normal pregnancy (Lundström, 1950), apparently indicating exhaustion of the available iron stores. This assumption is confirmed *ex iuvantibus*, as the symptoms of iron deficiency can be prevented by prophylactic administration of iron by mouth (Lundström, 1950; Ben-

STEAD & THEOBALD, 1952) or saccharated iron oxide intravenously (HAGBERG & LUNDSTRÖM, 1953).

Whereas a negative iron balance is evidently characteristic for the mother, both full term and premature infants have adequate iron stores at the time of birth, regardless of the maternal haemoglobin level. This has already been discussed at some length in chapter II.

Material. The original series consisted of 23 healthy mothers and their infants at delivery. Samples were not obtained in time from two of the women who therefore were excluded. The final, statistically treated series thus consisted of 21 women aged 16—43 years (average 26) and 23 infants with a birth weight varying between 2840—3870 g. In 4 5 of the series the pregnancy was the first or second. The sample from the mother was collected just as the child was born. The values for the infants refer to samples from the umbilical cord taken immediately after it was cut. The blood was allowed to drip freely into the iron-free test tube from the placental part of the cord.

Results. The results are recorded in table XII. The striking differences between mother and child, previously observed by other investigators, were confirmed.

TABLE XII

	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI TIBC per cent	SI/UIBC
Mother	21	98±6.7	470±15.3	21	0.3
Umbil. cord.....	23	173±6.9	259±10.5	70	2.1[1]

[1] 4 cases excluded where UIBC = 0. Median 2.0 (all cases).

The serum iron (SI) and the total iron-binding capacity (TIBC) in mother and child at parturition.

The average serum iron of the mothers was low but the difference from that of non-pregnant healthy women was not statistically significant. The TIBC, on the other hand, was significantly increased, and the saturation index and quotient significantly lowered, in comparison with non-pregnant values.

The serum iron level of the umbilical cord blood was in contrast significantly raised, whereas the TIBC was far below the average values of healthy adults. This difference was also statistically significant. The saturation index and quotient were the highest measured in healthy human subjects.

Discussion and conclusions. The serum iron levels of the mothers are high compared with the findings of VAHLQUIST (table XIII), but agree somewhat better with LAURELL's figures (table XIII) and those of SUNDELIN (1942) and LUNDSTRÖM (1950). The discrepancies cannot be satisfactorily explained.

TABLE XIII

Investigator	Mother			Umbil. cord		
	No. of cases	SI	TIBC M ± ε (M)	No. of cases	SI	TIBC M ± ε (M)
VAHLQUIST 1941	30	59	—	32	160	—
LAURELL 1947	SI 17 TIBC 25	80	446 ± 13	SI 20 TIBC 25	147	226 ± 10
HAGBERG 1953	21	98	470 ± 15.3	23	173	259 ± 10.5

The serum iron (SI) and the total iron-binding capacity (TIBC) in mother and child at parturition, a comparison with the results of other investigators.

TABLE XIV

Investigator	Time for sampling	No. of cases	TIBC gamma per cent
LAURELL 1947	P[2]	25	446
FAY *et al.* 1949	40th w.	9	583
RATH *et al.* 1950	P	21	354
VENTURA & KLOPPER 1951	30th—40th w.	25	453
MORGENTHAU *et al.*[1] 1952	P	48	>500
HAGBERG 1953	P	21	470

[1] See SMITH *et al.*, 1952. [2] P = at parturition.

The serum iron (SI) and the total iron-binding capacity (TIBC) in pregnant women at full term.

The finding of a markedly increased TIBC is consistent with the results of all investigators except RATH *et al.* (table XIV). They found strikingly low average values. Their series, however, also shows a tendency towards increased values during late pregnancy. The quantitative differences in TIBC vary greatly from one series to another. To some extent this fact may be explained by the limitations of methods based on the principles of SCHADE & CAROLINE (see chapter III). With an UIBC of the magnitude measured here even small deviations in the iron concentration of the test solution play a significant role. My values agree satisfactorily with those of LAURELL, who used a more accurate method.

Although the average TIBC in pregnant women at delivery is similar to that found in iron deficiency anaemia, it is questionable whether the raised level alone is a sign of iron deficiency; at least when combined with a normal serum iron level such an assumption is unjustified. It is interesting to note that prophylactic administration of intravenous iron (500—1000 mg) to healthy,

non-anaemic, pregnant women failed to depress the TIBC at delivery (Holly, 1951; Hagberg & Lundström, 1953). The increased TIBC seems therefore to be better explained as an expression of a regulation mechanism directing the iron to the fetus. In contrast to the apparently uninfluenced TIBC, low serum iron values were restored to normal on supplementing the oral iron (Lundström, 1950), indicating recovery from an iron deficiency state.

The serum iron and the TIBC of cord blood agree with the findings of Vahlquist (1941), Laurell (1947), and Smith *et al.* (1952). Some of the individual TIBC values were, however, approximate, as cord blood often contains large amounts of coloured substances (see p. 35).

Because of the high saturation index and quotient of infants at birth it is tempting to draw a parallel with conditions in which there are excessive iron stores, e.g. haemochromatosis, haemosiderosis, or haemolytic anaemias. The iron stores of the new-born are, however, not at all as unproportionally large (McCance & Widdowson, 1951) as was formerly believed, nor do the low TIBC and high saturation necessarily indicate excessively filled iron stores. They merely seem to signify that iron stores are being built up, i.e. that iron is being diverted from the fetal circulation to the depots. Thus all changes in both mother and child seem to promote a transfer of iron from the depots of the mother to the tissues of the fetus. This explains why even children of grossly iron deficient mothers do not show any signs of sideropaenia at birth (Strauss, 1933).

3. Healthy infants and children of all ages

It was previously stressed (chapter II) that the amount of available storage iron apparently changes more during infancy than at any other age. The investigations on healthy infants and children were therefore concentrated to the lowest age groups in order to establish the values corresponding to these rapid fluctuations in the metabolism of iron.

Material. The material consisted of 128 samples from 121 full term infants and children up to 3 years of age. Not less than 78 samples represented ages under six months. Most of the infants were patients from the Paediatric Clinic, Uppsala, chiefly healthy children of sick mothers, and social cases. A few of them were from a babies' home in the same city. Only infants whose red blood count was within the normal limits established by Vahlquist (1948) were included. No distinction was made between breast-fed and bottle-fed infants. Children who showed any signs of infection during the preceding week, or were suffering from diseases of the blood, were excluded. All samples were taken in the fasting state between 8 and 9 o'clock in the morning. In the age groups below one year all values refer to 'capillary' blood; in older children

TABLE XV

Age group	No. of cases	SI gamma per cent		TIBC gamma per cent		SI/TIBC per cent		SI−UIBC	
		M ± ε (M)[1]	S. D.	M ± ε (M)	S. D.	M ± ε (M)	S. D.	M ± ε (M)	S. D.
New-born (umbil. cord)	23	173 ± 6.9	33	259 ± 10.5	50	70 ± 4.9	23	2.1 ± 0.4[2]	1.6
½—2 mths.	26	142 ± 7.1	36	212 ± 6.6	34	69 ± 4.0	21	2.5 ± 0.4[3]	1.9
2—4 "	32	113 ± 5.6	31	308 ± 11.3	64	39 ± 2.5	14	0.7 ± 0.1	0.5
4—6 "	20	78 ± 6.1	27	360 ± 12.5	56	22 ± 1.9	9	0.3 ± 0.03	0.2
6—12 "	26	93 ± 6.5	33	394 ± 13.4	68	27 ± 2.4	12	0.3 ± 0.03	0.2
1—3 yrs.	24	99 ± 5.9	29	387 ± 9.7	47	26 ± 1.6	8	0.4 ± 0.03	0.2
3—7 "	21	124 ± 8.7	40	368 ± 9.6	43	34 ± 2.4	11	0.6 ± 0.06	0.3
7—14 "	28	119 ± 6.7	35	353 ± 7.6	40	34 ± 2.0	10	0.6 ± 0.05	0.3
Adults (both sexes)	54	130 ± 5.2	38	330 ± 4.9	36	40 ± 1.9	14	0.9 ± 0.1	0.7

[1] Mean ± standard error. S. D. = standard deviation.

[2] 4 cases excluded where UIBC = 0. Median 2.0 (23 cases).

[3] 4 cases excluded where UIBC = 0. Median 2.5 (26 cases).

The serum iron (SI) and the total iron-binding capacity (TIBC) at different ages during infancy and childhood.

the venous blood was taken from the cubital or femoral veins. With two exceptions sera from infants less than 15 days old were not included as they were usually found to be unsuitable for determination of the UIBC, owing to the presence in serum of large amounts of interfering substances during the period of physiological icterus (see p. 35).

The rest of the material consisted of 49 single samples from non-infected children representing all ages between 3 and 14 years. Most of the children were attending the Paediatric Clinic or the Child Psychiatric Clinic, and the others came from a children's home. Venous blood was collected as described above. Only cases with a normal blood picture have been included.

The whole series was divided into different age groups (see table XV) and evaluated statistically. No distinction was made between umbilical cord blood, 'capillary' blood, and venous blood.

Results. The results are recorded in table XV and sketched in figure 9.

The serum iron level fell from a maximum value at birth to a minimum before the end of the first half year. The difference between the level at birth and at the age of ½—2 months was statistically significant (31 ± 9.9), as was the difference between the latter group and infants of 4—6 months of age (64 ± 9.4). After this time the serum iron remained on a low level for the remainder of the first 2—3 years, showing only a slight increase if any; later it increased more rapidly. Thus the values in children of 3—7 years of age seemed to be higher (difference 25 ± 10.5) than those in the age group 1—3 years, and even agreed with the values in adult women.

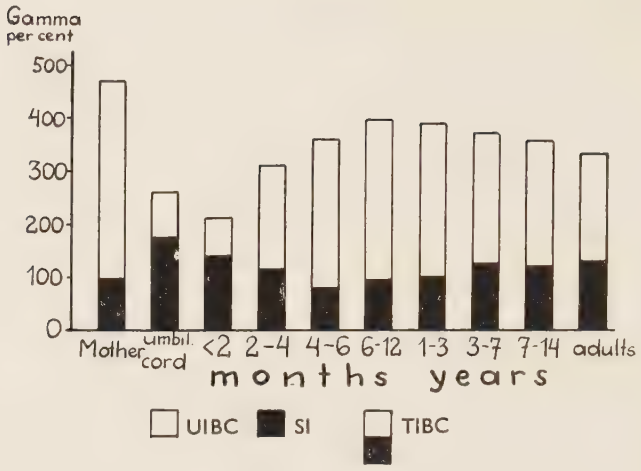

Fig. 9. *The normal series drawn schematically.*

The TIBC, already low at birth, fell further during the first two months. The difference between this and the figures for cord blood is statistically significant (difference 47 ± 12.4). After this initial lowering the TIBC rapidly rose, the increase being statistically significant by 2—4 months (difference 96 ± 13.1). The peak was reached in the age group 6—12 months, with an average value of 394 gamma per cent. This high level was found to be maintained during the next two years, when a successive fall to the adult level appeared to begin. The TIBC, however, seemed to return to normal more slowly than the serum iron. Thus the serum iron of the age group 3—14 years did not differ from the adult level, whereas the corresponding TIBC was significantly higher (difference 29 ± 7.7).

Apart from the first two months of life the TIBC followed the expected pattern, i.e. increased when the serum iron decreased. The maximum changes did not, however, correspond exactly in time, the lowest serum iron value appearing some months before the highest level of the TIBC.

As the serum iron and the TIBC fell relatively uniformly from the levels at birth to those at $\frac{1}{2}$—2 months of age, the saturation index and quotient remained practically unchanged. Thus the high transferrin saturation at birth was found to continue during the first two months of life. After this time the values fell steadily from the highest ever found in healthy human beings to the lowest. The minimum values were already reached at 2—4 months, and then continued on the same low level for the rest of the first two years. The increase which followed seemed to proceed successively until adulthood.

A few infants were examined more than once during the first six months

TABLE XVI

Sex Record, no.	Age, mths.	SI gamma per cent	TIBC gamma per cent	SI/TIBC per cent
♂ 658/52	1 1/4	140	240	58
	2	122	320	38
	3	78	420	19
	4	69	405	17
	4 3/4	37	405	9
♀ 107/52	1 1/2	179	280	64
	1 3/4	136	265	51
	3	134	405	33
	5 1/2	71	385	18
♀ 725/51	1	214	240	89
	2	104	250	42
	3	112	335	34
♀ 745/51	1	146	230	64
	3	108	355	31
♀ 348/51	1 3/4	188	190	100
	3	125	270	47
♀ 1600/51[1]	umbil. c.	138	225	61
	3	88	345	26
♀ 1591/51[1]	umbil. c.	130	280	47
	3	84	370	23
♂ 1535/51[1]	umbil. c.	158	285	55
	3	68	460	15

[1] Record number in the Obstetrical Department.

Repeated determinations of the serum iron (SI) and the total iron-binding capacity (TIBC) in infants during the first months of life.

of life, and these repeated analyses are given in table XVI. They confirm the results of the statistically analysed series.

The sex-differences at various ages were found to be inconsiderable. All results in the ages 3—14 years were evaluated statistically (table XVII). There was a tendency to an elevated TIBC in girls but no statistically significant differences were obtained. The higher values mentioned seemed to be due to random variation.

TABLE XVII

Group	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI/TIBC per cent M±ε (M)
Children, 3—14 years:				
Boys	25	118± 6.0	349± 9.1	35±2.1
Girls	24	124± 8.9	370± 7.3	34±2.2
Difference		6±10.7	21±11.7	1±3.0
Adults:				
Men	26	137± 6.3	321± 7.7	44±2.6
Women	28	123± 8.3	338± 5.9	37±2.6
Difference		14±10.4	17± 9.7	7±3.6

The sex-differences in children and adults.

TABLE XVIII

Hb gram per cent	No. of cases	SI gamma per cent M±ε (M)	TIBC gamma per cent M±ε (M)	SI/TIBC per cent M±ε (M)	SI/UIBC M±ε (M)
<11.5	29	87±4.7	403±10.6	22±1.2	0.29±0.2
11.5 and over	20	111±6.7	373±13.1	30±1.9	0.45±0.04
Difference		24±8.2	30±16.8	8±2.2	0.16±0.05

The serum iron (SI) and the total iron-binding capacity (TIBC) related to the haemoglobin level in 49 children aged between 6 months and 3 years.

The possible presence of slight iron deficiency anaemias in the series was checked. Children in the age groups with diminished iron stores (6 months—3 years) were divided into two sub-groups according to the haemoglobin level (table XVIII). It was found that those with haemoglobin values less than 11.5 gram per 100 ml had significantly lower serum iron values, saturation index, and quotient. The TIBC, however, did not differ significantly between the two sub-groups. Occasional infants among those with lower haemoglobin levels were found to have values quite in accordance with those found in iron deficiency anaemia.

Discussion and conclusions. The general tendency of the change in serum iron at different ages is in keeping with the findings of VAHLQUIST (1941), and there is also conformity with his corresponding figures for separate age groups (table XIX), if the diurnal variations are taken into consideration. Most of his observations in the ages six months and over refer to samples taken during the afternoon (VAHLQUIST, personal communication). The revised figures of MÖLLER & VAHLQUIST (1946) show that the corresponding morning values

TABLE XIX

VAHLQUIST 1941			SMITH *et al.* 1952			HAGBERG 1953		
Age group	No. of cases	SI gamma per cent	Age group	No. of cases	SI gamma per cent	Age group	No. of cases	SI gamma per cent
14 days	21	125	—	—	—	1/2—2 mths.	26	142
1— 6 mths.	24	89	1— 6 mths.	7	132	2— 6 mths.	52	99
6—12 mths.	23(25)	61[1](87)[2]	6—12 mths.	7	106	6—12 mths.	26	93
1—2 1/4 yrs.	39	58[1]	1— 2 yrs.	7	95	1— 3 yrs.	24	99
7 yrs.	60	103	2— 6 yrs.	9	116	3— 7 yrs.	21	124
11 yrs.	50	109	6—12 yrs.	13	127	7—12 yrs.	28	119

[1] Refers to samples taken during the afternoon.

[2] Revised morning values (MÖLLER & VAHLQUIST, 1946).

The serum iron values (SI) at various ages compared with the results of VAHLQUIST (1941) and SMITH *et al.* (1952).

lie on a level 20—25 gamma per cent higher. My figures for older children also tally with the findings of THOENES & ASCHAFFENBURG (1934), SCHÄFER (1940), and SMITH *et al.* (1952). These authors all give average values of about 120 gamma per cent.

Infants 1/2—2 months. This age group, covering the period of physiologically suppressed erythropoiesis and post-natal siderosis, has not previously been separately investigated with regard to the serum iron level. Although this is significantly diminished in comparison with the findings at birth, it must be noted that the level is still higher than at any other age in life. The TIBC, moreover, shows a significant fall, not formerly known. In this way the saturation index and quotient remain as high as at birth, and thus the same ratio between the serum iron and the TIBC was found at two distinct periods, both with continuous storing of iron going on, although the actual values were different. This clearly supports the hypothesis of LAURELL concerning the regulation of the plasma transport of iron.

Infants 2—6 months. During this period there is a successive return of full haematopoietic activity and simultaneously an increased need for iron in the bone marrow. The direction of the plasma iron transport turns from storage to mobilization. At the same time the serum iron falls to its lowest values, and the TIBC successively rises and passes normal adult levels. The saturation index and quotient rapidly reach minimum values close to those found in iron deficiency anaemia.

Infants and children 6 months—3 years. Due to continuous mobilization of iron during this stage the reserves become more or less depleted. Infants and children of this age are therefore particularly vulnerable to even the smallest

additional strain on the iron balance. Thus simple nutritional iron deficiency anaemias are commoner during this period than at any other age. Even in healthy children there are trends well compatible with a slight iron deficiency anaemia. The microcytosis, the low haemoglobin, and the lowered serum iron levels, in particular have been mentioned.

The problem has been studied *ex iuvantibus* by MÖLLER & VAHLQUIST (1946) and HORAN (1950). Each administered prophylactic doses of iron to healthy infants for six weeks and three months respectively. MÖLLER & VAHLQUIST examined the children at 6—8 months of age and found no difference between the treated group and the controls, either in the red blood picture or in the serum iron level. They concluded that the lower physiological limits are difficult to fix but that the following average blood values are quite physiological for healthy infants after the first half year of life: haemoglobin 12—12.5 gram per cent, red cells 4.5 million, colour index 0.85, serum iron 85 gamma per cent. HORAN made her examinations when the children were 12 months of age. In spite of small differences her findings suggest on the other hand that increasing iron intake influences the haemoglobin, red cell count, and haematocrit reading.

SMITH and associates (1952) investigated the alterations in serum iron and TIBC at certain ages (table XX) and found the same tendency as VAHLQUIST (1941) and myself, i.e. a lowered serum iron level and a markedly elevated TIBC. However, the successive rise to a peak soon after the first half year of life did not appear in their results, probably because of their smaller series and fewer age groups. These authors stress that their findings in infants of 6 months—2 years of age suggest an iron deficiency state despite the absence of anaemia.

My findings (table XVIII) of significantly lowered values for serum iron, saturation index, and quotient, in children with low but normal haemoglobin

TABLE XX

SMITH *et al.* 1952			HAGBERG 1953		
Age group	No. of cases	TIBC	Age group	No. of cases	TIBC
5— 8 days	8	262	1/2— 2 mths	26	212
1— 6 mths	7	412	2— 6 mths	52	328
6—12 mths	7	429	6—12 mths	26	394
1— 2 yrs	7	414	1— 3 yrs	24	387
2— 6 yrs	9	395	3— 7 yrs	21	368
6—12 yrs	13	340	7—12 yrs	28	353

The total iron-binding capacity (TIBC) in healthy infants and children, a comparison with the values of SMITH *et al.* (1952).

levels suggest that some cases with iron deficiency anaemia are included in the series. This is supported by the results in single cases. These facts indicate that the lower limit of normal haemoglobin values is difficult to fix and probably varies from case to case.

The lowered serum iron, the elevated TIBC, and the decreased saturation index and quotient, imply a heightened ability of the organism to mobilize iron. The comparatively small iron reserves which are characteristic of and probably normal in these ages may explain the need for such a measure. As depleted iron stores are replenished very slowly in adults even on adequate iron therapy (HASKINS *et al.*, 1952), it seems reasonable to suppose that this process will be still more lengthy in growing subjects, and that prophylactic doses of iron by mouth might not suffice either to restore or to maintain the iron depots of a rapidly growing child. Such a mechanism might well explain the failure to restore the serum iron level in series such as that of MÖLLER & VAHLQUIST, and is in keeping with VAHLQUIST's assertion (1941) that a lowered serum iron level must be regarded as physiological in the ages in question.

Children over three years of age. After 2—3 years the iron balance becomes successively stabilized, and the stores replenished through an adequate diet. At the same time the serum iron and the TIBC approach the adult values. However, the TIBC is still significantly higher than the normal adult level, which implies a lower saturation index and quotient. This may even here be explained by the extra demand for iron to provide for growth. JASIŃSKI (1951) also believes in a high frequency (50 per cent) of slight iron deficiency anaemias. He interpreted elevated oral iron tolerance curves as evidence of a real lack of tissue iron even in cases with a normal or high serum iron level. Such a generalisation would appear somewhat sweeping, as uncomplicated iron deficiency states with high serum iron levels do not exist as far as is known. Because of a more labile and vulnerable iron balance all transition forms are, on the other hand, probably common in children, from a physiologically increased demand and a latent iron deficiency state to a slight but obvious iron deficiency anaemia.

Summing up it may be said that the changes in the TIBC during infancy and childhood strongly support the assumption that the transferrin level is of importance in the storage and mobilization of iron. Thus there is a close parallelism between iron storage and low TIBC, and between depletion of the depots and elevated TIBC. There would also usually appear to be a mutual relationship between the transferrin level and the magnitude of the iron stores, but the chief determining factor seems to be the direction of the iron flow.

4. Iron deficiency anaemia

Iron deficiency anaemia is much commoner during late infancy and early childhood than during any other age period. This fact is apparently due to the small stores of iron mentioned above, which do not sustain even apparently slight stresses. The common direct causes of iron deficiency are rapid growth, inadequate and monotonous gruel diet, or ordinary anorrhexia. Predisposing factors are prematurity, twin birth, early clamping of the umbilical cord, and severe iron deficiency in the mother.

In older children severe iron deficiency anaemia does occur (VAHLQUIST, 1941), but in Scandinavian countries it is nowadays rare. Slight forms are commoner, and are usually often connected with abnormal eating habits or postinfectious anorrhexia. Nutritional factors are probably also important causes of the high frequency of iron deficiency anaemia among mentally deficient children (HAGBERG, 1953b).

Infants and children representing the various categories mentioned above

TABLE XXI

Record no.	Sex Age yrs.	Diagnosis	Hb gram per cent	Colour index	SI gamma per cent	TIBC gamma per cent	SI/TIBC per cent
424/52	♂ 1	nutritive iron deficiency anaemia	7.2	0.48	21	475	4
161/51	♂ 1½	nutritive iron deficiency anaemia	7.7	0.77	40	475	8
52/52	♂ 1	nutritive iron deficiency anaemia + spastic colitis	9.4	0.89	76	500	15
404/51	♀ 1	praematurity + iron deficiency and megaloblastic anaemia	8.0	0.65	64	485	13
820/52	♂ 1	iron deficiency and megaloblastic anaemia	6.5	0.73	42	530	8
637/51	♂ 8	idiopathic thrombocytopaenia + hypoproteinaemia + iron deficiency anaemia	9.7	0.81	45	440	10
254/51	♀ 13	iron deficiency anaemia (essential hereditary)	6.1	0.48	31	515	6
—	♂ 15	iron deficiency anaemia (essential)	5.1	0.55	34	555	6
572/51	♂ 2	cœliac disease + iron deficiency anaemia	10.0	0.84	28	465	6
851/52	♀ 11	ulcerous colitis + iron deficiency anaemia	5.4	0.53	25	500	5
858/52	♂ 1	iron deficiency anaemia + idiocy	7.0	0.47	42	500	8
67/52	♀ 2	" " " + "	8.3	0.61	40	515	8
64/52	♂ 4	" " " + "	8.1	0.64	44	500	9
201/51	♂ 5	" " " + "	7.5	0.70	78	510	15
512/51	♂ 5	" " " + oligophrenia .	9.4	0.71	117!	460	25
200/51	♂ 6	" " " + idiocy	10.0	0.73	80	500	16

Infants and children with iron deficiency anaemia.

TABLE XXII

Age	No. of cases	SI gamma per cent M ± ε (M)	TIBC gamma per cent M ± ε (M)	SI/TIBC per cent M ± ε (M)	SI/UIBC M ± ε (M)
<5 years	9	44	494	9	0.10
5 and over	7	59	498	12	0.14
All ages	16	50 ± 6.5	496 ± 7.1	10 ± 1.4	0.12 ± 0.02

The serum iron (SI) and the total iron-binding capacity (TIBC) in children with iron deficiency anaemia.

have been investigated with regard to changes in the serum iron and the TIBC, and are reported below.

Material. The series consisted of 16 cases (see table XXI), all except one being patients in the paediatric wards. They were aged between 1 and 15 years, and all were free from infection.

Results. Most of the cases had a low serum iron, and all of them a high TIBC and a decreased saturation index and quotient. The lowest TIBC (440 gamma per cent) was found in a case with simultaneous hypoproteinaemia. There was no definite correlation between the degree of the changes in TIBC and the fall in haemoglobin level.

Table XXII shows the statistical evaluation of the series. All values differ significantly from those of healthy children in all age groups. The changes seemed to be of the same magnitude independent of age.

Discussion and conclusions. The results agree perfectly with the findings of SMITH *et al.* (1952) in a similar series. These authors concluded that the pattern of low serum iron and elevated UIBC represents depletion of the iron stores irrespective of a normal or anaemic red blood picture.

Determinations of both the serum iron and the TIBC give a more accurate idea of the need for iron in the tissue stores. It is, however, important to stress that *both* values are changed in a true iron deficiency state. This fact is of particular importance in children aged 6 months to 3 years, as healthy children with normal serum iron sometimes have a TIBC quite in keeping with values found in iron deficiency anaemia (table XXIII). Iron therapy would, however, always seem to be indicated when the TIBC is elevated, as this alteration apparently expresses a heightened readiness to mobilize and absorb iron. The presence, but not the extent, of a true iron deficiency is, however, revealed by a raised TIBC only when combined with a low serum iron.

Replenishment of the iron stores is characterized by a successively rising serum iron level and a falling TIBC (fig. 10). The return to normal does not seem to be complete until the haemoglobin is also fully restored. Supernormal

TABLE XXIII

Record no.	Sex Age mths.	Hb gram per cent	Colour index	SI gamma per cent	TIBC gamma per cent	SI/TIBC per cent
966/51	♀ 8	11.8	1.15	106	525	20
988/52	♀ 18	11.2	1.05	140	490	29
759/51	♀ 11	11.5	0.97	206	475	43
(P. B.)	♂ 12	11.1	0.82	94	475	20
(B. S.)	♂ 8	10.9	0.85	99	470	21
835/52	♂ 8	11.1	0.98	92	450	20

Healthy infants and children aged 6 months—3 years with a total iron-binding capacity (TIBC) over 450 gamma per cent in spite of normal haemoglobin levels, and serum iron values (SI) on or over the average level for the age.

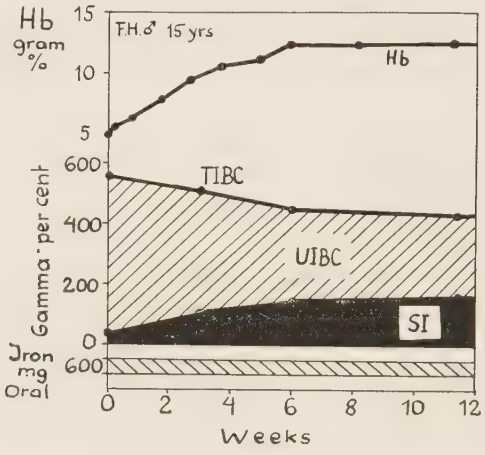

Fig. 10. *The changes in serum iron and total iron-binding capacity during oral iron therapy in a boy aged 15 years with an iron deficiency anaemia of unknown origin.*

values for the TIBC are still found long afterwards, probably indicating a continued readiness to mobilize and metabolize iron. This is in keeping with the very slow restoration of the iron stores found even with extensive oral iron treatment (HASKINS *et al.*, 1952).

5. Acute infectious diseases

The metabolism of iron is severely disturbed in infectious states. The mechanism of this and the changes in the serum iron have been studied by HEILMEYER (1937—41) and CARTWRIGHT, WINTROBE, and associates (1946—52)[1];

[1] For references see the review by CARTWRIGHT & WINTROBE (1952).

and on children by Vahlquist (1941), Schäfer (1940—49), and others. Investigations on the changes in the iron-binding capacity have been made by Holmberg & Laurell (1945), Laurell (1947), Cartwright & Wintrobe (1949), Rath & Finch (1949), Smith *et al.* (1952), and Braunsteiner *et al.* (1952).

Acute infections are characterized by a low serum iron and a more or less diminished TIBC. Thus the TIBC is altered during infections just as in states with increased iron stores (fig. 1, p. 16) in spite of serum iron levels corresponding to those found in iron deficiency. Iron tolerance tests show that iron is eliminated from the blood plasma faster than normally. The normocytic anaemia which often accompanies infectious conditions seems to be due neither to changes in the TIBC nor to iron deficiency. The changes in the iron values and the anaemia of infection are best regarded as manifestations of a non-specific inflammatory reaction in the body. The same findings are also met with in experimentally produced aseptic inflammations (Robsheit-Robbins & Whipple, 1936; Cartwright & Wintrobe, 1949), in various toxic conditions, especially uraemia (Laurell, 1947), and in most cases of malignant tumours (Laurell, 1947).

The anaemia of infection seems to be caused mainly by impaired production of haemoglobin (Wintrobe *et al.*, 1947) combined with disturbed erythropoiesis (Hemmeler, 1946). Iron normally taken up in the bone marrow cannot be used there, and instead is diverted to reticulo-endothelial cells in the liver and spleen (Schäfer, 1942). Increased sternal marrow haemosiderin has also been observed (Rath & Finch, 1948). In septic conditions there is also probably an increased rate of destruction of red cells (see for instance Cartwright & Wintrobe, 1952; Hagberg, 1952) which gives a further surplus of iron. There is thus an increased deposition of iron in the usual storage organs (Roth *et al.*, 1951), but this has little to do with normal storage. In fact, the reticulo-endothelial cells seem to have such a demand for iron that it disappears from the plasma at an increased rate, resulting in hypoferraemia. That this is the result of reticulo-endothelial activity is stressed by Cartwright *et al.* (1950), who demonstrated in dogs that hypoferraemia due to sterile turpentine abscesses could be abolished by a 'blockade' of the RES. Why this system removes iron from plasma during infection is not clear but the same authors (1951) suggest that the adrenal cortex may be concerned.

Whereas the principal changes in the serum iron and the iron-binding capacity during infection are well known and the cause of infectious hypoferraemia has been extensively studied, the factors influencing the extent and the rate of the changes in the TIBC have been far less elucidated. In this investigation the relation of these factors to the severity and duration of infec-

tion has been studied, and also the speed of the changes in comparison with the change in the serum iron.

Material. The TIBC was studied on samples collected from 82 children of all ages between 5 months and 14 years, at different stages of various acute infections. Children with incidental iron deficiency anaemia have been excluded as far as possible. All values refer to the morning fasting state. In most cases a single sample was taken, and was collected either when pyrexia was still present or just as it was beginning to abate. Some of the cases were examined repeatedly during the course of the disease and convalescence.

For statistical analysis the series was divided into two main groups. Children with pharyngitis, tonsillitis, sinusitis, uncomplicated lymphadenitis, and bronchitis, were referred to 'slight infections'; rheumatic fever, primary tuberculosis with pyrexia, pneumonia, septic lymphadenitis, suppurative otitis, and some other diseases of a similar degree of severity, were grouped as 'severe infections'.

Results. It appears from table XXIV that children with various infections of 2—3 days duration evidently have a TIBC which seems to be little or not at all changed. On the other hand the serum iron values were generally found to be low even on the first days of pyrexia.

Table XXV demonstrates that many of the cases with severe infectious diseases were found to have a lowered TIBC on the fourth to sixth day of the disease, whereas the corresponding values during slight infections were found

Table XXIV

Record no.	Sex Age yrs.	Diagnosis	Day of pyrexia	SI gamma per cent	TIBC gamma per cent
223/51	♂ 1	pharyngobronchitis	2	66	455
165/51	♀ 5	pharyngotonsillitis	2	70	430
941/52	♂ 3	pharyngitis	2	36	385
1185/50	♂ 2	pharyngobronchitis	2	36	375
115/51	♀ 6	bronchopneumonia	2	44	370
210/51	♀ $^1/_2$	suppurative otitis media	2	64	365
1097/51	♀ 6	acute pyelitis	2	52	345
748/52	♀ 4	otitis media + pharyngobronchitis	3	34	340
840/51	♀ 8	tonsillitis	3	20	340
812/51	♀ 10	tonsillitis	2	42	325
46/51	♂ 3	pharyngitis	2	66	320
78/51	♂ 3 $^1/_4$	asthmathic bronchitis	3	64	305
865/51	♂ 12	bronchopneumonia	3	48	300
420/52	♀ 13 $^1/_2$	bronchopneumonia	2	37	295

The serum iron (SI) and the total iron-binding capacity (TIBC) in 14 non-anaemic infants and children with acute infections and pyrexia of 2—3 days duration.

Table XXV

Record no.	Sex Age yrs.	Diagnosis	Day of disease	ESR mm	TIBC gamma per cent
186/51	♂ 3	pharyngobronchitis	6	31	380
967/51	♂ 5 1/2	rhinopharyngitis	6	43	355
461/51	♀ 8	pharyngitis	6	25	355
84/52	♀ 4 3/4	tonsillitis + cervical lymphadenitis	6	34	350
831/51	♀ 6	acute mesenteric lymphadenitis	6	31	350
145/51	♂ 3 1/2	pneumonia	4	44	340
115/51	♀ 6	bronchopneumonia	6	9	340
1158/50	♂ 8 3/4	atypical pneumonia	5	35	335
978/52	♂ 8	pneumonia	4	49	320
717/51	♂ 7 1/4	pharyngobronchitis	5	19	320
840/51	♀ 8 1/4	tonsillitis	5	30	315
812/51	♀ 10 1/2	tonsillitis	6	46	315
978/51	♀ 7	atypical pneumonia	6	48	310
275/52	♂ 6 1/2	rheumatic fever	6	63	295
262/52	♀ 7 3/4	rheumatic fever	5	66	290
407/51	♂ 3 1/4	pharyngitis	4	41	285
88/51	♂ 4 1/4	pneumonia	4	48	270
420/52	♀ 13 1/2	bronchopneumonia	4	43	265
365/52	♀ 8 1/4	pneumonia	5	54	255
182/51	♀ 10 1/2	rheumatic fever	4	41	245
630/51	♀ 4 1/4	primary tuberculosis	4	55	235
105/51	♂ 13 3/4	acute pancarditis	4	65	230
846/52	♀ 4 1/2	tuberculous pleurisy	6	36	175

The total iron-binding capacity (TIBC) in 23 children aged 3—14 years with various infectious diseases. Sampling was done 4—6 days after the beginning of the pyrexia.

to be within normal limits but with a somewhat lower average level. Approximately the same results were found in cases with infectious diseases of 1—3 weeks duration, as can be seen in table XXVI; markedly lower values were not found in spite of the longer duration of infection.

The results of repeated estimations of TIBC during infection in individual cases (fig. 11 and table XXVII) confirm the findings in tables XXIV—XXVI. They also show that the return of the TIBC to normal seems to occur gradually within the week following recovery, that is to say usually somewhat more slowly than the serum iron (fig. 12).

Children suffering from infectious diseases with a protracted course seemed to have a TIBC which remained quite constant after an initial fall (fig. 13). This level varied from case to case and seemed to be determined by the severity of the disease rather than the actual diagnosis. Several children with lobar

TABLE XXVI

Record no.	Sex Age yrs.	Diagnosis	Week of disease	ESR mm	TIBC gamma per cent
1141/51	♀ 6	maxillar sinusitis + bronchitis	1 1/2	20	370
508/51	♀ 5 1/2	whooping cough + bronchopneumonia	1 1/2	41	350
967/51	♂ 5 1/2	pharyngobronchitis	1 1/2	43	345
489/51	♂ 3 1/2	pharyngolaryngitis	2	13	345
978/51	♀ 7	atypical pneumonia	1 1/2	48	340
163/51	♂ 8	atypical pneumonia	1	30	315
392/51	♂ 7	acute cervical lymphadenitis	1	45	310
1158/50	♂ 8 3/4	atypical pneumonia	2	40	310
538/52	♀ 10 3/4	rheumatic fever	1 1/2	81	300
312/52	♂ 4 3/4	acute mesenteric lymphadenitis	1	41	295
492/51	♂ 13	bronchopneumonia	1 1/2	45	290
825/51	♂ 7 3/4	bronchopneumonia	2	19	285
356/51	♂ 12 1/2	maxillar sinusitis	1 1/2	37	280
23/51	♀ 10 1/4	rheumatic fever	2	64	270
668/51	♀ 11 1/4	Löffler's syndrome	1 1/2	25	260
87/51	♂ 5 1/2	acute cervical lymphadenitis	1 1/2	62	245
920/52	♀ 8 1/4	rheumatic fever	3	60	235
417/52	♀ 7 1/4	primary tuberculosis	2	66	235
1169/50	♂ 6 3/4	exsudative pleurisy	>3	35	225
615/51	♀ 7 1/2	atypical pneumonia	2	45	200

The total iron-binding capacity (TIBC) in 20 children aged 3—14 years with various infectious diseases of 1—3 weeks duration.

or bronchopneumonia but in good general condition had a TIBC within normal limits, whereas in those severely ill it was often found to be low. The lowest values obtained (TIBC = 175 gamma per cent) were in a 2-year-old boy critically ill with an atypical pneumonia, and a 4-year-old girl with a severe tuberculous pleurisy and toxic signs.

Tables XXVIII and XXIX record the statistical analysis of the effect of the severity and duration of infectious diseases upon the TIBC. It is obvious (table XXVIII) that severe infections reduce the TIBC far below the level of healthy children in the same age group. The difference (75 ± 9.5 gamma per cent) is statistically significant. The mean value in 'slight infections' is also less than that in healthy children. The difference (23 ± 8.1 gamma per cent) is statistically probable but might be significant in a larger series.

Table XXIX demonstrates that the fall in TIBC due to severe infections was significantly greater than that in slight infectious diseases. The difference was apparent during the first four days of the disease, but was not statistically significant until the infection had lasted five days or more. Compared with

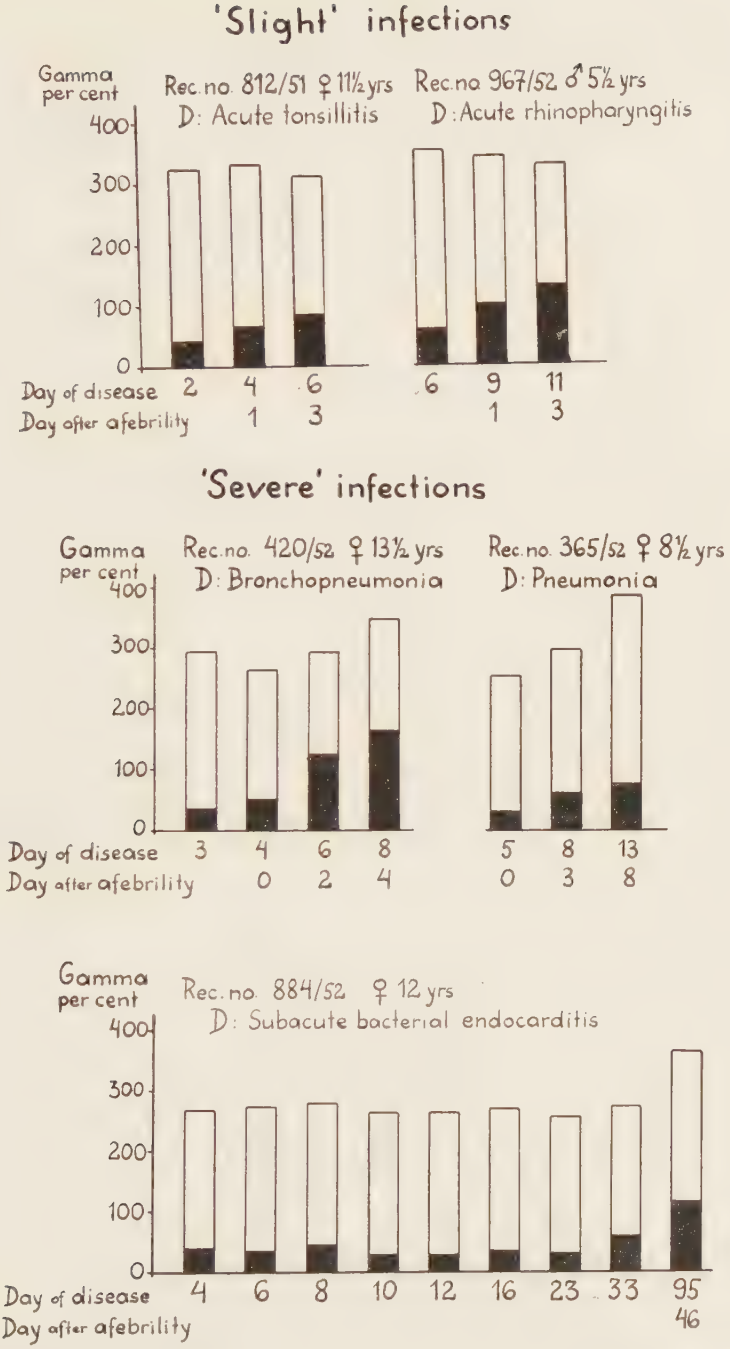

Fig. 11. *Representative examples of infected children in whom repeated determinations of the serum iron and the total iron-binding capacity were made.*

TABLE XXVII

Sex Age Rec. no.	Day of disease	Day after afebrility	Day of ACTH treatment	ESR mm	SI gamma per cent	TIBC gamma per cent
	6			63	67	295
♂	9	0	3		67	310
	12	3	6	59	62	305
6 1/2 yrs.	15	6	9		61	385
	20	11	14	32	130	415
275/52	41	32	—	16	100	420
	56	47	—	11	83	405
	19	2	4	60	82	235
♀	22	5	7		52	285
8 yrs.	25	8	10	30	90	295
38/52	30	13	15	17	90	350
	70	53	—	8	109	345
♀	5	0	1	66	84	290
7 3/4 yrs.	10	5	6	32	116	355
262/52						
♀	4			41	62	245
10 1/2 yrs.	13	6	8	55	72	310
182/51						

The changes in serum iron (SI) and total iron-binding capacity (TIBC) in patients with rheumatic fever in remission during ACTH-treatment.

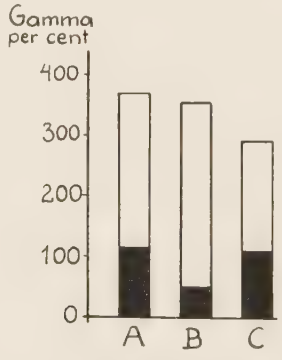

Fig. 12. *The fall in serum iron takes place during the first day of infection and is rapidly restored on recovery. The changes in TIBC occur more slowly.*

A. 73 healthy children aged 1—14 years.

B. 14 children with acute infections and pyrexia for three days or less (table XXIV).

C. 14 children on the first day of afebrility after severe infectious diseases of five days duration or more.

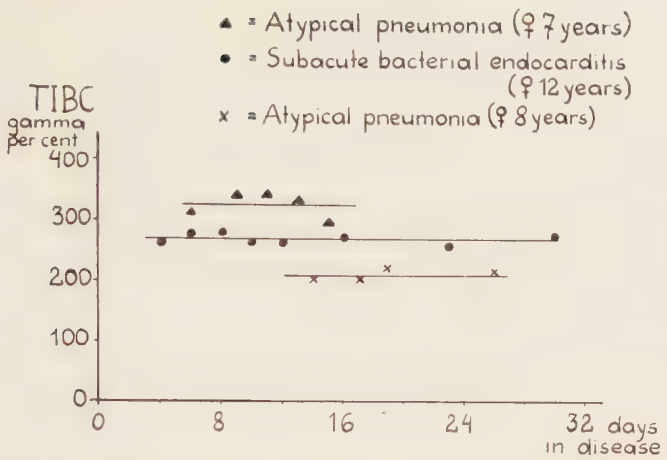

Fig. 13. *Three girls followed throughout the course of infectious diseases. After an initial fall the TIBC remains on a fairly constant level until recovery.*

TABLE XXVIII

Children 1—14 yrs.	No. of cases	TIBC gamma per cent M ± ε (M)	Decrease in comparison with healthy subjects
Healthy	73	368 ± 5.3	—
Slightly infected	33	345 ± 6.1	23 ± 8.1
Severely infected	49	293 ± 7.9	75 ± 9.5

The decrease in the total iron-binding capacity (TIBC) during slight and severe infectious diseases in comparison with the values in healthy children.

TABLE XXIX

Day of disease	Age yrs.	Slight infections		Severe infections		P
		No. of cases	TIBC gamma per cent M ± ε (M)	No. of cases	TIBC gamma per cent M ± ε (M)	
1—4	1— 5	11	349	8	332	>0.05
	5—14	5	355	9	300	>0.05
	1—14	16	351 ± 9.7	17	315 ± 15.1	>0.05
5—ω	1— 5	8	351	11	282	0.01—0.001
	5—14	9	331	21	279	0.01—0.001
	1—14	17	340 ± 7.6	32	280 ± 8.4	<0.001
	All children	33	345 ± 6.1	49	293 ± 7.9	<0.001

The changes in the total iron-binding capacity (TIBC) during slight and severe infectious diseases of varying duration.

Table XXX

ESR mm	Age group (yrs.)	No. of cases	TIBC gamma per cent M ± ε (M)
<30	<5	12	349
	5 and over	8	346
	All ages	20	348 ± 12.7
35—50	<5	17	319
	5 and over	20	306
	All ages	37	312 ± 8.7
>50	<5	12	300
	5 and over	10	275
	All ages	22	289 ± 10.2

The relation between the decrease in the total iron-binding capacity (TIBC) and the increase in the erythrocyte sedimentation rate (ESR) in children with infectious diseases.

healthy children of the same age there was, however, a statistically significant fall in the TIBC during the first four days of severe infectious disease.

The results recorded in table XXX confirm once more that the severity of the infection is of importance. Cases with an erythrocyte sedimentation rate (ESR) of more than 50 mm were found to have a lower TIBC than those in whom it was less than 30 mm. The difference (59 ± 16.3) is statistically significant. There seems, however, to be no consistent correlation between a high sedimentation rate and a low TIBC. Some cases had an only moderately increased sedimentation rate but a markedly lowered TIBC (see tables XXV and XXVI).

No consistent connection between the changes in the TIBC and the degree of pyrexia was found. Some of the lowest TIBC values were observed in cases with moderate pyrexia of relatively short duration, whereas a boy aged ten with a reticulum cell sarcoma (rec. no. 47/52) and continuous fever of about 39—40° C for over one month had a low normal TIBC (285 gamma per cent).

Discussion and conclusions. It has been confirmed that acute infectious diseases diminish the TIBC. The changes in children do not seem to be more pronounced than in adults, to judge from the results of other observers. In the first place the extent of the changes seems to depend upon the severity of the disease. Slight infections such as common pharyngitis usually bring about insignificant changes in the TIBC, whereas prostrating infections are most often associated with considerably lowered values. This is not contradicted by the wide range of variation in the TIBC found in cases with the

same diagnosis, as the lowest values generally seem to be found in patients most severely ill. Similarly, most cases with pyrexia and a markedly increased erythrocyte sedimentation rate have a lowered TIBC, and here again there is no definite correlation.

In spite of a predominance of slight infections of short duration and severe infections with a longer course, the results give an idea of the changes in the TIBC due to the duration of the infection. It thus seems to fall steadily from the onset, reaching a level of equilibrium within the first week. The rate of this initial fall and its total extent apparently depend in the first place on the severity of the infection, but individual factors may also be of importance. Once the level of equilibrium is attained the TIBC seems to remain fairly constant until the infection abates. There is then a gradual return to normal, probably lasting several days.

It has not been possible to determine whether the post-infectious rise in the TIBC temporarily exceeds the pre-infectious level. Such an alteration seems reasonable in the light of the reversal of the plasma iron transport during recovery. From the cases investigated it was impossible to evaluate fully the importance of the pre-infectious level in determining the degree of the subsequent fall in TIBC. Very low values have, however, been observed in infected cases in whom a definite iron deficiency existed, which suggests that the infection was predominant. A similar dominance of changes due to infection also applies to the serum iron (Vahlquist, 1941).

The results support the assertion of Cartwright & Wintrobe (1949) that the drop in transferrin during infection does not cause the hypoferraemia. This is finally proved by the fact that the fluctuation in serum iron seems to occur more rapidly than that in the TIBC. Thus definitely low serum iron values were found during the second day of illness, when the TIBC was in all cases still within normal limits, and the average was only slightly lowered. On the other hand in many cases the serum iron apparently returns to normal by the first day of afebrility, whereas the TIBC is still low. Thus the changes in the serum iron and the TIBC during infections seem to be independent.

The cause of the hypoferraemia during infectious states seems to be the increased demand of iron by the RES, the reason for which is hard to explain. The fall in the TIBC seems to be better interpreted as a regulative mechanism during a reversed iron metabolism than as a result of disturbed protein synthesis. It is tempting to assume that the fall is an expression of the increased deposition of iron in the storage organs during infection, the extent depending on the severity of the disturbance as the amount of iron available for deposition is probably determined by the degree of erythropoietic depression and the extent of break-down of red cells.

SUMMARY

Changes in the iron metabolism are reflected in the plasma and can be estimated from fluctuations in the iron-transporting plasma protein, transferrin, and its amount of attached iron, the serum iron.

This study refers to investigations on the serum iron and the total iron-binding capacity of the transferrin in about 300 infants and children. Special emphasis has been laid on the physiological changes occurring during infancy, when the amount of storage iron changes more than at any other time in later life. The iron stores, satisfactory at birth, increase during the first two months, but thereafter fall successively, remaining small during the next few years.

Methods

In estimating the total iron-binding capacity, the iron-saturated fraction (the serum iron) and the unsaturated fraction (the unsaturated iron-binding capacity) were determined independently and added together.

For the determination of the serum iron a modification of the method of Vahlquist (1941) was used and found sufficiently accurate. Only 0.3 ml of serum was needed. Instead of filtering, the sample was centrifuged after precipitation of the proteins.

The unsaturated iron-binding capacity was determined according to the method of Rath & Finch (1949), which is based on the fact that the salmon-red iron-transferrin colour deepens on fractionated addition of iron until full saturation is attained. This method was found to have definite limitations. It was unreliable in lipaemic sera or sera with discoloration or severe disturbance of the plasma protein pattern. If the concentration of bilirubin exceeded 3—4 mg per cent the estimation could not be satisfactorily performed, whereas slight haemolysis did not interfere with the result. In spite of its disadvantages the method was, however, found to be accurate enough for the special purposes of this investigation, a fact proved by iron tolerance tests.

A simple 'one stage measurement' of the total increase in extinction after supersaturation with iron was found to give too unreliable values, probably owing to the presence of variable amounts of substances interfering with the development of the iron-transferrin colour. Possibly due to the same mechanism an average introductory iron enrichment of 15 gamma per 100 ml was found to be necessary to obtain any increase in extinction at all.

Clinical investigations

1. The average serum iron level in 54 healthy adults (26 men and 28 women) was 130 ± 5.2 gamma per cent, and the corresponding total iron-binding capacity 330 ± 1.9 gamma per cent. These figures served as basic values for judging the results obtained in infants and children.

2. The serum iron of the *umbilical cord* at parturition was as high as 173 ± 6.9 gamma per cent, whereas *the total iron-binding capacity was 259 ± 10.5, i.e. significantly less than in healthy adults. The changes parallel a stage of active storage of iron*, and are in striking contrast to the pattern in the serum of the mothers, in whom there is mobilization of iron from the depots.

3. *Infants ½—2 months* of age are in a state of physiologically suppressed erythropoietic activity, which implies a diminished need and hence *increased storage of iron*. They showed *a still lower total iron-binding capacity*, 212 ± 6.6 gamma per cent, and an average serum iron of 142 ± 7.1 gamma per cent, i.e. significantly less than at birth but still high. It is notable that *the relative saturation with iron was the same as in cord blood.*

4. *Infants aged 2—6 months*, the period of return to full erythropoietic activity during which *increasing amounts of iron are mobilized from the depots*, had a falling serum iron (113 ± 5.6 gamma per cent at 2—4 months, and 78 ± 6.1 at 4—6 months) and *a rising total iron-binding capacity* (308 ± 11.3 gamma per cent at 2—4 months, and 360 ± 12.5 at 4—6 months).

5. The serum iron values in *infants and children of ½—3 years* remained low, with an average of about 95 gamma per cent, and *the total iron-binding capacity was markedly elevated*, with an average of about 390 gamma per cent. *It is at these ages that the iron stores are minimal*, and the children are particularly vulnerable to even small additional strains on the iron balance. The values obtained put in relation to the haemoglobin level also suggested that some cases with slight iron deficiency anaemia were included in a 'normal' series in spite of haemoglobin values within accepted limits.

6. The values in *older children successively approached adult levels*, which were attained more rapidly by the serum iron than by the total iron-binding capacity.

7. A series of 16 children with *iron deficiency anaemia* had an average serum iron of 50 ± 6.5 gamma per cent, and a total iron-binding capacity of 496 ± 7.1 gamma per cent.

8. The general conclusion was that in infants and children the level of the total iron-binding capacity and its saturation with iron closely reflects the probable direction of the plasma iron transport, i.e. the storage and mobilization of iron, and thus usually also the size of the iron depots. The findings clearly support LAURELL's *hypothesis concerning the regulation of the plasma transport of iron.*

9. The metabolism of iron is disturbed during *infection*. The disturbance is revealed by a simultaneous fall in the serum iron and total iron-binding capacity. This pattern of change deviates from the physiological outlined above.

From studies in 82 children suffering from various acute infections it was found that *the extent and rate of the fall in total iron-binding capacity was mainly related to the severity of the infection*. It developed successively during the first week, and reached a level of equilibrium which remained constant in infections with a protracted course. The return to normal seemed to occur gradually within the week following recovery.

It was also found that *the changes in serum iron generally occurred more rapidly than those in the total iron-binding capacity*. This confirmed earlier assumptions that the lowering of the total iron-binding capacity does not cause the hypoferraemia of infection.

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Résumé

La capacité du serum de lier le fer chez les nourrissons et les enfants

Les changements dans le métabolisme du fer se reflètent dans le plasma et peuvent être déterminés à partir des oscillations dans le plasma protéineux, la transferrine (qui transporte le fer), et dans la masse de fer qui y est attaché, le fer sérique.

Cette étude concerne les recherches, conduites chez environ 300 nourrissons et enfants, sur le fer sérique et la capacité totale qu'a la transferrine de lier le fer. On a mis spécialement l'accent sur les changements physiologiques qui se produisent au cours de l'enfance lorsque la masse disponible de fer change plus qu'à n'importe quel autre moment de la vie. Les réserves de fer, suffisantes à la naissance, augmentent au cours des deux premiers mois, mais, après, diminuent peu à peu et restent faibles au cours des premières années suivantes.

Méthodes

En estimant la capacité totale de lier le fer, la fraction saturée de fer (le fer sérique) et la fraction non saturée (la capacité de lier le fer non saturée) furent déterminées indépendamment, et additionnées. Pour déterminer le fer sérique, on s'est servi d'une modification de la méthode Vahlquist (1941) qui s'est révélée suffisamment exacte. Il ne fallut que 0.3 ml de serum. Au lieu du filtrage, une centrifugation fut faite après la précipitation des protéines.

La capacité de lier le fer non saturée fut déterminée d'après la méthode Rath et Finch (1949) qui se base sur le fait que la couleur rouge saumon de la transferrine du fer se fonce après une addition graduée de fer jusqu'à ce que pleine saturation soit atteinte. On a constaté, que cette méthode a des limites définies. Elle était inexacte dans les serums lipémiques et les serums avec décoloration ou perturbation considérable du type du plasma protéineux. Si la concentration de bilirubine dépassait 3 à 4 mg pour cent, l'estimation ne pourrait être évaluée de manière satisfaisante, tandis que une hémolyse légère ne modifie pas le résultat. Cependant, malgré ses désavantages, la méthode se révéla suffisamment exacte pour le but que nous poursuivions: ce qui a été prouvé par des recherches de la tolérance du fer.

La "simple mesure d'une phase" de l'augmentation totale en extinction après supersaturation de fer se trouva donner des résultats trop douteux,

probablement à cause de la présence de masses différentes de substances qui interfèrent avec le développement de la couleur transferrine du fer.

Probablement dû au même mécanisme, un enrichissement préliminaire de fer de 15 γ par 100 ml en moyenne se trouva nécéssaire pour obtenir n'importe quelle augmentation en extinction.

Recherches cliniques

1. Le niveau moyen du fer sérique chez 54 adultes (26 hommes et 28 femmes) en bonne santé fut 130 ± 5.2 γ% et la capacité correspondante totale de lier le fer 330 ± 4.9 γ%. Ces chiffres ont servi de valeurs de base pour juger les résultats obtenus chez enfants et nourrissons.

2. Le fer sérique du *cordon ombilical* s'élevait à 173 ± 6.9 γ% à la naissance, tandis que *la capacité totale de lier le fer fut 259 ± 10.5 γ%, c-à-d considérablement moins que chez des adultes en bonne santé. Les changements sont parallèles à une phase d'emmagasinement actif de fer* et contrastent violemment avec le type du serum maternel où il y a une libération de fer à partir des dépôts.

3. *Les nourrissons âgés de deux semaines à deux mois* sont dans un état d'activité érythropoiétique physiologiquement représsive, ce qui implique un besoin diminué, et, désormais *une augmentation du stock de fer*. Ils montraient *une capacité totale de lier le fer encore plus basse*, 212 ± 6,6 γ%, et un fer sérique de 142 ± 7.1 γ% en moyenne, c-à-d une moyenne considérablement moindre qu'a la naissance mais pourtant élevée. Il est à remarquer que *la saturation de fer relative était la même que dans le sang du cordon.*

4. *Les nourrissons âgés de 2 à 6 mois*, période de retour à une pleine activité érythropoiétique pendant laquelle des *masses de fer croissantes sont libérées des dépôts*, avaient un fer sérique décroissant (113 ± 5.6 γ% de 2 à 4 mois, et 78 ± 6.1 γ% de 4 à 6 mois) et *une capacité totale de lier le fer progressive* (308 ± 11.3 γ% de 2 à 4 mois, et 360 ± 12.5 γ% de 4 à 6 mois).

5. Les valeurs du fer sérique chez *les nourrissons et chez les enfants âgés de six mois à trois ans*, restaient basses, environ 95 γ% en moyenne, et *leur capacité totale de lier le fer était remarquablement élevée*, environ 390 en moyenne. *C'est à cet âge que les réserves de fer sont infimes*, et que les enfants sont spécialement vulnérables même aux moindres surcharges de la balance du fer. Les valeurs obtenues mises en relation avec le niveau d'hémoglobine montraient aussi que quelques cas d'anémie ferriprive légère étaient inclus dans une série "normale" malgré des valeurs d'hémoglobine qui restent dans des limites acceptées.

6. Les valeurs chez *des enfants plus âgés s'approchaient peu à peu des niveaux d'adultes* qui étaient plus vite atteints par le fer sérique que par la capacité totale de lier le fer.

7. Une série de 16 enfants *d'anémie ferriprive* avait un fer sérique de 50 ± 6.5 γ% en moyenne, et une capacité totale de lier le fer de 496 ± 7.1 γ%.

8. La conclusion générale fut que chez les nourrissons et chez les enfants, le niveau de la capacité totale de lier le fer, et sa saturation de fer reflètent de près la direction probable du transport du fer du plasma, c-à-d emmagasinement et libération de fer, ainsi qu'ordinairement aussi le volume des dépôts de fer. Les découvertes supportent avec précision l'hypothèse Laurell en ce qui concerne la régularisation du transport de fer dans le plasma.

9. Le métabolisme du fer est troublé au cours d'une *infection*. La perturbation se révèle par une réduction simultanée dans le fer sérique et par une réduction de la capacité totale de lier le fer. Ce type de changement diffère du comportement physiologique esquissé ci-dessus.

Par des études conduites sur 82 enfants souffrant de diverses infections aiguës, il est apparu que *l'extension et la rapidité de la réduction de la capacité totale de lier le fer eurent surtout rapport au degré de gravité de l'infection*. La réduction s'accentua peu à peu durant la première semaine et atteignit un niveau d'équilibre qui resta constant lors d'infections d'une durée assez longue. Le retour à l'état normal semble s'être fait graduellement dans la semaine qui a suivi la guérison.

On a trouvé aussi que *les changements dans le fer sérique en général ont lieu plus rapidement que ceux de la capacité totale de lier le fer*. Cela confirma les hypothèses précédentes que la réduction de la capacité totale de lier le fer ne cause pas l'hypoferrémie d'infection.

Zusammenfassung

Das Eisenbindungsvermögen des Serums bei Säuglingen und Kindern

Die Änderungen des Eisenumsatzes spiegeln sich im Plasma ab und können von den Schwankungen des eisentragenden Plasmaproteins, das Transferrin, und seinem Betrag an gebundenem Eisen bestimmt werden.

Diese Arbeit bezieht sich auf Versuche am Serumeisen und am totalen Eisenbindungsvermögen des Transferrins bei ungefähr 300 Säuglingen und Kindern. Besonderer Nachdruck wurde auf die physiologischen Schwankungen während der Kindheit gelegt, wenn die Menge des zur Verfügung stehenden Depoteisens mehr als je im späteren Leben wechselt. Die Eisenvorräte, welche bei der Geburt befriedigend sind, nehmen während der zwei ersten Monate zu, werden aber allmählich erschöpft und bleiben in den nächsten darauffolgenden Jahren gering.

Methoden

Zur Bestimmung des totalen Eisenbindungsvermögens wurden die eisengesättigte Fraktion (das Serumeisen) und die ungesättigte (das ungesättigte Eisenbindungsvermögen) unabhängig voneinander bestimmt und nachher zusammen addiert.

Zur Bestimmung des Serumeisens wurde eine Abwandlung der Methode von VAHLQUIST (1941) angewandt und für genügend exakt befunden. Es wurden nur 0,3 ml Serum hinzugefügt. Anstatt zu filtrieren, wurde nach der Fällung der Proteine zentrifugiert.

Das ungesättigte Eisenbindungsvermögen wurde nach der Methode von RATH & FINCH (1949) bestimmt, welche sich auf die Tatsache gründet, dass die rote Lachsfarbe des Eisentransferrins sich bei schrittweisem Zusatz von Eisen vertieft bis die Sättigung erreicht wird. Dagegen war sie bei Fettsera oder bei verfärbten Sera oder bei Sera mit schweren Störungen des Plasmaeiweissgerüstes unzuverlässig. Wenn die Konzentration des Bilirubins 3—4 mg Prozent überstieg, konnte die Bestimmung nicht genau ausgeführt werden, während eine schwache Hämolyse das Resultat nicht störte. Trotz dieser Nachteile wurde die Methode für die speziellen Absichten dieser Versuche für genügend genau gefunden, ein Faktum, das durch Eisenverträglichkeitstests bewiesen wurde.

Eine einfache "einmalige Messung" der totalen Zunahme der Extinktion nach Übersättigung mit Eisen wurde für zu unzuverlässig befunden. Wahrscheinlich hängt das von veränderlichen Mengen von Substanzen ab, welche die Entwicklung der Eisentransferrinfarbe hemmen. Möglicherweise abhängig vom selben Mechanismus ist die Tatsache, dass eine einleitende Durchschnittseisenbereicherung von 15 γ per 100 ml als notwendig gefunden wurde, um Überhaupt eine Zunahme der Extinktion zu erhalten.

Klinische Untersuchungen

1. Der Durchschnitt des Serumeisenspiegels bei 54 gesunden Erwachsenen (26 Männer und 28 Frauen) war $130 \pm 5{,}2$ γ % und das entsprechende totale Eisenbindungsvermögen $330 \pm 4{,}9$ γ %. Diese Bilder dienen als grundlegende Werte für die Beurteilung der bei Säuglingen und Kindern erhaltenen Resultate.

2. Der Serumeisengehalt der *Nabelschnur* war bei der Geburt $173 \pm 6{,}9$ γ %, während *das totale Eisenbindungsvermögen* $259 \pm 10{,}5$ γ % *war, d.h. bedeutend niedrigere Werte als die bei Erwachsenen aufwies. Die Veränderungen gehen parallel mit einer aktiven Eisenspeicherungsstelle* und stehen in auffallendem Kontrast zu dem Bild in dem Serum der Mütter, mit deren erhöhte Eisenmobilisation aus den Depots.

3. *Säuglinge im Alter von $^1/_2$—2 Monaten* befinden sich in einem Stadium von physiologisch gesenkter erythropoietischer Aktivität, was ein geringeres Bedürfnis an Eisen und damit eine *erhöhte Eisenspeicherung* bedeutet. Sie zeigten *ferner eine Senkung des totalen Eisenbindungsvermögens*, 212 ± 6,6 γ%, und einen Serumeisendurchschnittswert von 142 ± 7,1 γ%, d.h. bedeutend niedriger als bei der Geburt, aber noch hoch. Es ist auffallend, dass *die entsprechende Sättigung mit Eisen dieselbe wie in der Nabelschnur war.*

4. *Kinder im Alter von 2—6 Monaten*, d.h. wenn die volle erythropoietische Aktivität langsam wiederkommt und *eine wachsende Eisenmenge von den Depots mobilisiert wird*, hatten einen Abfall des Serumeisens (113 ± 5,6 γ% im 2.—4. Monat, und 78 ± 6,1 γ% im 4.—6. Monat) und ein *zunehmendes totales Eisenbindungsvermögen* (308 ± 11,3 γ% im 2.—4. Monat und 360 ± 12,5 γ% im 4.—6. Monat).

5. Die Serumeisenwerte bei *Säuglingen und Kindern zwischen $^1/_2$—3 Jahren* blieben niedrig mit einem Durchschnittswert von ungefähr 95 γ%. *Ihr totales Eisenbindungsvermögen war auffallend hoch*, der Durchschnittswert war ungefähr 390. *Es ist in diesen Altersgruppen bei denen die Eisenspeicherung minimal ist* und die Kinder besonders empfindlich auch für kleine Schwankungen des Eisengleichgewichts sind. Setzt man die erhaltenen Werte in Beziehung zu dem Hemoglobinspiegel, so deuten sie an, dass einige Fälle mit einer leichten Eisenmangelanämie in die "normalen" Serien aufgenommen wurden, trotzdem die Hämoglobinwerte innerhalb annehmbarer Grenzen liegen.

6. *Die Werte der älteren Kinder näherten sich allmählich den Werten der Erwachsenen* und wurden vom Serumeisen schneller als vom totalen Eisenbindungsvermögen erreicht.

7. Eine Serie von 16 Kindern mit *Eisenmangelanämie* hatte einen Serumeisendurchschnittswert von 50 ± 6,5 γ% und ein totales Eisenbindungsvermögen von 496 ± 7,1 γ%.

8. Es wurde der allgemeine Schluss gezogen, dass bei Säuglingen und Kindern die Höhe des totalen Eisenbindungsvermögens und seine Sättigung mit Eisen die wahrscheinliche Richtung des Plasmaeisentransports nahe wiederspiegelt, d.h. die Aufspeicherung und Mobilisation von Eisen und damit also gewöhnlich den Umfang der Eisendepots.

9. Der Eisenstoffwechsel ist während *Infektionen* gestört. Die Störung äussert sich, unter anderen Dingen, durch einen Abfall des Serumeisens und zur gleichen Zeit eine Senkung des totalen Eisenbindungsvermögens. Dieses Bild von Veränderungen weicht von dem weiter oben beschriebenen physiologischen Bild ab.

Bei an 82 Kindern gemachten Untersuchungen, die an verschiedenen akuten Infektionen litten, wurde gefunden, dass *der Umfang und das Mass des Abfalles des totalen Eisenbindungsvermögens hauptsächlich zu der Schwere*

der Infektion in Beziehung standen. Er entfaltete sich allmählich während der ersten Woche und erreichte einen Gleichgewichtsspiegel, welcher sich bei Infektionen mit protrahiertem Kurs konstant hielt. Die Rückkehr zu normalen Werten schien im Bereich der ersten Woche nach der Wiederherstellung schrittweise einzufallen.

Es wurde auch gefunden, dass die *Veränderungen des Serumeisens im allgemeinen schneller vor sich gingen als diejenigen des totalen Eisenbindungsvermögens.* Das bestätigt frühere Behauptungen, dass die Senkung des totalen Eisenbindungsvermögens nicht die Ursache der Hypoferremia der Infektion sein kann.

Resumen

La capacidad de fijación de hierro del suero en lactantes y niños

Los cambios del metabolismo del hierro se reflejan en el plasma y pueden valorarse de las fluctuaciones de la proteina transportadora del hierro, la transferrina, y de la cantidad de hierro a ella ligada, a saber el hierro del suero.

Este estudio se refiere a investigaciones sobre el hierro del suero y la capacidad de fijación total de hierro de la transferrina en 300 lactantes y niños aproximadamente. Se ha puesto un interés especial en los cambios fisiológicos durante la infancia, tiempo en el que la cantidad de reservas de hierro disponibles varia más que en edades más avanzadas. Las reservas de hierro, satisfactorias al nacer, aumentan durante los dos primeros meses y van disminuyendo después sucesivamente, durante los primeros próximos años.

Métodos

Para determinar la capacidad total de fijación de hierro, se analizaron la fracción de hierro saturada (el hierro suérico) y la insaturada (la capacidad de fijación de hierro insaturada) cada una por si sola y sumándose después.

Para analizar el hierro suérico se usó una modificación del método de Vahlquist (1941) encontrándose suficientemente exacta. Solo se añadieron 0,3 ml de suero y en lugar de filtrar se centrifugó después de la precipitación de las proteinas.

La capacidad de fijación de hierro insaturada se determinó según el método de Rath & Finch (1949), que se basa en el hecho de que el color rojo asalmonado de la transferrina férrica se intensifica con la adición graduada de hierro hasta que se ha alcanzado la saturación total. Se encontró que este método tiene ciertas limitaciones. De este modo resultó ser inexacto en sueros lipémicos, sueros decolorados o en graves trastornos del armazón de la proteina plasmática. Si la concentración de la bilirubina excedía unos 3—4 mg por

ciento, el análisis no podía llevarse a cabo satisfactoriamente, mientras que una hemolisis débil no estorbaba el resultado. A pesar de estas desventajas el método se encontró lo suficientemente exacto para los propósitos especiales de estas investigaciones, hecho probado a través de tests de tolerancia férrica.

Se encontró que la simple medida de una fase del aumento total de la extinción después de la supersaturación con hierro daba resultados inexactos. Es probable que esto se deba a la presencia de cantidades variables de substancias que inhiben la evolución del color de la transferrina férrica. Posiblemente debido al mismo mecanismo se encontró que es necesario un enriquecimiento de hierro medio de 15 γ por 100 ml para llegar a obtener un aumento de la extinción.

Investigaciones clínicas

1. El promedio de hierro suérico en 54 adultos sanos (26 hombres y 28 mujeres) fué de 130 ± 5,2 γ% y la capacidad total de hierro respectiva 330 ± 4,9 γ%. Estas figuras sirven de valores básicos para juzgar los resultados obtenidos en lactantes y niños.

2. El hierro suérico del *cordón umbilical* en el parto fué de 173 ± 6,9 γ%, mientras que la *capacidad total de fijación de hierro fué de* 259 ± 10,5 γ%, *es decir mostró valores más bajos que en adultos sanos.* Los cambios van paralelos a un almacenamiento activo de hierro y están en contraste violento con el cuadro del suero de las madres donde hay una liberación de hierro de los depósitos.

3. *Lactantes de* $^1/_2$*—2* meses de edad se encuentran en un estado de actividad eritropoyética fisiológica baja, lo que significa una necesidad disminuida de hierro y de este modo un *aumento de los depósitos.* Muestran *además una disminución de la capacidad total de fijación de hierro,* 212 ± 6,6 γ% y un promedio de hierro suérico de 142 ± 7,1 γ%, es decir marcadamente más bajo que al nacer, pero sin embargo alto. Es notable que la saturación relativa de hierro fuera la misma que la del cordón umbilical.

4. *Los lactantes de 2—6 meses,* es decir cuando la actividad eritropoyética total vuelve sucesivamente y *se moviliza un aumento creciente de hierro de los depósitos,* mostraron una baja del hierro suérico (113 ± 5,6 γ% de los 2—4 meses y 78 ± 6,1 γ% de los 4—6 meses) y un *aumento de la capacidad total de fijación de hierro* (308 ± 11,3 γ% de los 2—4 meses y 360 ± 12,5 γ% de los 4—6 meses).

5. Los valores de hierro suérico de *lactantes y niños entre* $^1/_2$*—3 años* se mantuvieron a un nivel más bajo con un promedio de 95 γ% aproximadamente. *Su capacidad total de fijación de hierro fué extraordinariamente elevada* y el promedio de unos 390. Justamente en estas edades los depósitos de hierro son minimales y los niños particularmente sensibles incluso a pequeñas

alteraciones adicionales del balance del hierro. Si se ponen los valores obtenidos en relación con la hemoglobina, estos indican también que algunos casos con una anemia hipoférrica débil fueron incluidos en series "normales" a pesar de mostrar valores hemoglobínicos dentro de límites aceptables.

6. *Los valores en los niños de más edad se aproximan sucesivamente a los de los adultos* que fueron alcanzados más rápidamente por el hierro suérico que por la capacidad total de fijación de hierro.

7. Una serie de 16 niños con *anemia hipoférrica* mostraron un valor promedio de hierro suérico de $50 \pm 6{,}5\ \gamma\%$ y una capacidad total de fijación de hierro de $496 \pm 7{,}1\ \gamma\%$.

8. Se sacó la conclusión de que el nivel de la capacidad total de fijación de hierro y su saturación de hierro en lactantes y niños reflejan la dirección probable del transporte de hierro plasmático, es decir el almacenamiento y la movilización de hierro así como la magnitud de los depósitos de hierro. Los resultados apoyan claramente la hipótesis de Laurell referente a la regulación del transporte del hierro plasmático.

9. El metabolismo del hierro está alterado durante las *infecciones* y entre otras cosas se revela por una baja en el hierro suérico y al mismo tiempo por una disminución de la capacidad total de fijación de hierro. Este cuadro de variaciones difiere del cuadro fisiológico más arriba descrito.

De estudios de 82 niños que sufrían distintas infecciones agudas se sacó la conclusión de que la *extensión y la velocidad de la baja de la capacidad total de fijación de hierro estuvo principalmente relacionada con la gravedad de la infección*. Se desarrolló sucesivamente durante la primera semana y alcanzó un equilibrio que se mantuvo constante en infecciones con un curso protrahido. La vuelta a valores normales pareció ocurrir gradualmente durante la semana siguiente al restablecimiento.

Se encontró también que los *cambios del hierro suérico ocurren generalmente con más rapidez que aquellos de la capacidad total de fijación de hierro*. Esto confirma las suposiciones anteriores de que la baja de la capacidad total de fijación de hierro no es la que causa la hipoferremia de infección.

II

Intravenous Iron, its Plasma Transport, Toxic Reactions, and Use in Paediatrics

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1. Intravenous Iron Therapy and its Application in Paediatrics: A Short Review.
2. Intravenous Iron Therapy in Pediatrics. (Also published in Acta Pædiat. *40*: 519, 1951.)
3. The Iron-binding Capacity of Serum in Different Forms of Iron Poisoning. (Also published in Acta Soc. Medic. Upsaliensis *58*: 56, 1953.)
4. Experimental Investigations on Saccharated Iron Oxide. (Also published in Acta Soc. Medic. Upsaliensis *58*: 73, 1953 together with Vahlquist and Dickie.)
5. The Effect of Large Single Doses of Saccharated Iron Oxide on Antibody Production in Children. (Also published in Nord. Med. *49*: 252, 1953, in Swedish with an English summary.)

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Intravenous Iron Therapy and its Application in Paediatrics: A Short Review

INTRODUCTION

The limited capacity of human plasma to complex-bind ionized iron prevented for a long time the practical use of intravenous iron therapy. Even doses of about 10 mg of iron injected in dialysable form gave unpleasant symptoms indicating, as we now know (HOLMBERG & LAURELL, 1945), that the total iron-binding capacity (TIBC) had been exceeded. It is true that colloidal iron preparations, e.g. ferric hydroxide (GOETSCH *et al.*, 1946) had been tried and found to be tolerated in huge amounts. They nevertheless gave severe and alarming toxic reactions during the course of the injection, possibly due to instability of the colloid. The introduction of the saccharated iron oxides, which are usually well tolerated, has given intravenous iron therapy a widespread application. The theoretical explanation of the tolerance of single doses of up to 1000 mg of iron is shown schematically in fig. 1. It is obvious that huge iron concentrations in serum can be tolerated, provided that the TIBC is not exceeded by ionized iron (HAGBERG, 1951).

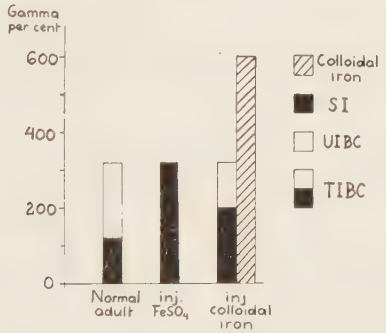

Fig. 1. *Schematic figure illustrating the difference between dialysable iron and colloidal iron in their relation to the iron-binding protein in the blood.* (SI = serum iron, UIBC = unsaturated iron-binding capacity, TIBC = total iron-binding capacity).

The preparation and its properties

Many saccharated iron oxides have been marketed during recent years. My investigations were performed with 'Intrafer' (Pharmacia), identical with the English 'Ferrivenin' (Benger). Like other preparations of this kind it

is very polydispersed and has a calculated molecular weight of more than 50000.

Turbidimetric measurements (Granath, 1952), and dialysis (Hagberg, Vahlquist & Dickie, 1953b), showed the complex to be stable *in vitro* within a wide range of pH (>11—6). Investigations on the magnitude of the unsaturated iron-binding capacity (UIBC) after injections of saccharated iron oxide (Hagberg, 1953a) to a certain extent supported these findings.

Recovery of colloidal iron on analysis of human serum

It was shown (Hagberg *et al.*, 1953b) that only 40—45 per cent of the iron in saccharated iron oxide is recovered with the usual serum iron technique from serum dilutions made *in vitro*. A nearly complete yield was, however, achieved by increasing the acidity, or by extending the reaction time with the hydrochloric acid and simultaneously raising the temperature. Even with these precautions, however, no more than about 60 per cent of the calculated amount of iron was recovered from samples collected from patients 5—10 minutes after injection of saccharated iron oxide. The rest of the iron had probably not disappeared from the blood but presumably remained among the packed red cells.

The plasma transport and the management of saccharated iron oxide in the body

In spite of iron concentrations far exceeding the TIBC, the transferrin was often found to be only partially saturated after intravenous injections of saccharated iron oxide (Hagberg, 1951, 1953a). Thus it was concluded that this form of iron evidently appears in the blood in a complex and mainly unchanged form. An initial fall in the UIBC was, however, thought to depend upon the liberation of a small fraction of ionized iron, possibly owing to a lowering of the stability of the saccharated iron oxide *in vivo*.

In the hours following injection a continuous increase in the degree of saturation was found, the mechanism of which could not be explained, although experiments showed that it could not be due to a passive splitting off of iron ions, i.e. by a further lowered stability of colloid remaining in the blood.

The concentration of colloidal iron in the blood fell rapidly during the hours following injection. In single adults with iron deficiency anaemia it had apparently left the blood within 5—6 hours, but in other cases a small fraction still seemed to be present after 24 hours.

Like other colloidal or suspensoid substances, saccharated iron oxide is taken up from the blood by reticulo-endothelial cells (Cappel, 1930; Andersson, 1950), which have a pronounced affinity for this colloid. Thus earlier it

was often used in animal experiments to block the RES. In the reticulo-endothelial cells the iron is apparently converted from a finely particulated to a soluble form, and is redistributed to the depots, mainly the parenchymal cells of the liver. This process is believed to be rather slow, taking days or weeks.

Therapeutic effect

All observers agree that the therapeutic effect of intravenous saccharated iron oxide in iron deficiency anaemia is generally very good and in most cases surprisingly rapid. Experiences in infants and children are uniform, the results of many observers pointing in the same direction (HAGBERG, 1951; DICKSTEIN *et al.*, 1952; STRANSKY & DAUIS-LAWAS, 1952; BRAHAM *et al.*, 1952; and SCHÖNER, 1952). The average haemoglobin increase varied between 0.15—0.55 gram per cent per day! The variations apparently depended to a large extent on the severity of the anaemias in the different series. Slow remission curves were sometimes found (HAGBERG, 1951) and may be related to a complicating protein deficiency. BRAHAM *et al.* (1952) also obtained good results in iron deficiency anaemias complicated by severe infections. However, in anaemia of infection without a preceding iron deficiency, intravenous iron was useless (KUHN *et al.*, 1950).

Due to their constant requirements of iron for growth, children are probably slower than adults in replenishing the exhausted iron stores. It is therefore especially important to pay attention to the impoverishment of the iron stores when calculating the optimum amount of iron to be injected. Recent experience has shown the formula presented in my first paper to be unsatisfactory. It should be revised to

$$5 \times \text{kg body-wt.} \times \text{haemoglobin deficiency (g-\%)} = \text{mg iron needed.}$$

About 50 per cent of the iron here is calculated for replenishment of the stores, which is in conformity with the opinion of BROWN *et al.* (1950).

It has been suggested (HAGBERG, 1951; STRANSKY & DAUIS-LAWAS, 1952) that children usually tolerate even very large single doses of saccharated iron oxides well, and probably better than adults. Thus children of 1—3 years were beneficially treated by single injections of up to 10 mg per kg body-weight. Calculating from the formula above it is, however, usually undesirable to replace the whole iron deficit in one injection. One possible alternative is to give the iron in a slow intravenous drip with 5 per cent glucose in those cases where repeated injections are difficult to perform.

Side effects

In spite of improved preparations toxic reactions do occur. The various saccharated iron oxides marketed all give excellent therapeutic results but differ in the frequency and type of general toxic reactions. Thus immediate toxic states with nausea, vomiting, lumbar pain, fall in blood pressure, and collapse, seem to be the most common side effect with German preparations (see for instance GOLDECK *et al.*, 1952), but seem to occur very seldom after injections of 'Ferrivenin'. One fatal case of this type has, however, recently been described by BARRIT & SWAIN (1953). The commoner side effects of the preparation used are as follows.

Immediate. During or some minutes after the injection a few patients developed slight flushing and coughing, symptoms quite similar in type to those seen after dialysable iron salts though considerably slighter. Vascular reactions due to a liberation of free iron ions (HAGBERG, 1953a) or precipitation of iron-protein aggregates (WOLF & STILLE, 1952; SCHWIETZER, 1952) are possible explanations. These slight reactions are probably equivalent to the immediate haemodynamic collapses which evidently constitute the most unpleasant side effect.

BROWN *et al.* (1950) found a transitory leucopaenia five minutes after injection. It disappeared within thirty minutes. WHITE *et al.* (1952) thoroughly explored this phenomenon. They concluded that it was due to a non-specific colloidal effect, well-known after injections of other colloidal substances; it has nothing to do with agranulocytosis, a parallel drawn by FJELLMAN (1952).

WHITE *et al.* (1952) also noted an increased coagulability of the blood immediately after injection, an observation which should be kept in mind when treating older patients with impaired cardiac function. It probably has the same pathogenesis as the leucopaenia mentioned above.

Finally, venous cramps during the course of the injection have also been reported by many observers. They may be quite painful but are more often insignificant.

1—5 hours after injection. The real toxic reactions following injections of 'Intrafer' ('Ferrivenin') appeared, with few exceptions (e.g. LIBRACH, 1953; BARRIT & SWAIN, 1953), 1—5 hours after the injection. The clinical picture was generally characterized by chills, pyrexia, nausea, and in severe cases collapse. Though infrequent and generally mild such reactions cannot be neglected.

There is a relation, which is not, however, fixed, between the appearance of toxic reactions and the size of the dose. It was found that the risk in infants and children was greater when doses exceeding 10 (!) mg per kg bodyweight were used. Occasionally, however, toxic reactions were encountered with remarkably low doses in adults (HAGBERG, 1953a).

The mechanism of these toxic reactions has been much debated. The discussion has often been confusing, as the various findings and hypotheses refer to different preparations of saccharated iron oxide. Moreover, most animal experiments have been carried out with doses far exceeding those used in clinical practice.

Three hypotheses deserve special attention.

Exceeding of the total iron-binding capacity is a tempting theoretical explanation put forward by some authors. It has, however, been shown that the toxic reactions are unconnected with exceeding of the saturation limit in serum (Hagberg, 1953a).

Haemodynamic disturbances due to precipitated iron-protein thrombi occluding various capillary systems are the most likely cause of the immediate collapses reported by German investigators (Stille, 1952; Schwietzer, 1952; Wolf & Stille, 1952). It is more questionable if the same mechanism can also explain the type of general reaction occurring later. The clinical pictures with chills and pyrexia as the commonest signs do not fit in with such an assumption, but the collapses might have the same pathogenesis.

Irritation or overloading of the reticulo-endothelial system is a third possible explanation first suggested by Ramsey (1950), who pointed out the resemblance to general reticulo-endothelial reactions, e.g. that seen in cold haemoglobinuria. His assumption seems to be a possible explanation of the obviously increased frequency of typical toxic signs seen in a group of children who received simultaneously intravenous saccharated iron oxide and subcutaneous toxoid as triple vaccine (Hagberg, 1953a and c). Findings of this type might well be related to a strong irritation or overloading of the RES by the saccharated iron oxide itself or by successively precipitated and phagocytosed iron-protein aggregates.

Late manifestations. Little is known of the late results of parenteral administration of large doses of iron. Practically all authors warn against artificially overloading the iron depots. Animal experiments (Polson, 1928; Cappel, 1930; Andersson, 1950; Brown *et al.*, 1950) and a few observations in man (Holly, 1951; Hagberg, 1951) after massive total doses showed an excess of haemosiderin in the storage organs but no cirrhosis, fibrosis, or other signs of parenchymatous damage. The period of observation is, however, still too short to allow definite conclusions. The decisive point seems to be whether a profusion of haemosiderin itself is or is not directly injurious to the tissues, and here the data are scanty. The haemosiderin is probably neither the sole nor the major cause of parenchymatous fibrosis, but may be an important aggravating factor (Clough, 1952). Especially in hypoproteinaemia sudden attempts at massive replenishment of depleted iron stores seem to be contraindicated (Schwietzer, 1951, 1952), because abnormal stor-

age probably occurs in the absence of sufficient amounts of protecting proteins. In states with impaired liver function it is also possible that a rapid substitution of the iron reserves may be injurious to the less resistant parenchymatous cells. Overloading the tissues with iron may thus imply a potential danger which should be avoided.

Influence on the reticulo-endothelial system

A massive uptake of colloidal iron may lead to a 'blockade' of the RES in test animals, and produce deficiency symptoms. Thus enzymatic functions and antibody production have been thought to be temporarily impaired. Although 'Intrafer' ('Ferrivenin') was found to be an effective 'blocking' agent in animal experiments (Gabrieli, 1952) it had no depressant effect on the antitoxin production in children who received simultaneously single injections of saccharated iron oxide and immunization against diphtheria (Hagberg, 1953c).

Indications for saccharated iron oxide in paediatrics

Since the response of patients with iron deficiency anaemia to oral iron is usually good there are few real indications for giving children intravenous iron. The range of application is still more limited by the above mentioned restriction in hypoproteinaemia and hepatic failure, as coeliac disease and ulcerative colitis, two important indications, are often complicated by one or both of these disturbances. Bearing this in mind it is, however, expedient to administer saccharated iron oxide to iron-deficient children who

1. are unable to derive full benefit from oral iron,
2. cannot tolerate oral iron therapy without severe gastro-intestinal disturbances,
3. do not tolerate irritation of the gastro-intestinal mucosa.

Sometimes it may also be desirable to precede oral therapy by an initial single dose of intravenous iron in cases of severe but uncomplicated nutritional iron deficiency anaemia. By doing so an improvement in the patient's general condition can often be achieved, and the haemoglobin restored more quickly than otherwise.

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Acknowledgements

These investigations were carried out in the Paediatric Department of the University Hospital, Uppsala, at the suggestion of the head of the Clinic, Professor Bo Vahlquist. My debt of gratitude to him is considerable for his deep interest in my work, for all his valuable advice and support during the course of the investigations, and for the wide paediatric and haematological knowledge he has put at my disposal.

My thanks are also due to Docent Carl-Bertil Laurell, Professor Gunnar Dahlberg, Professor Per Erik Lindahl, Professor Jan Waldenström, Docent Ragnar Berfenstam, Docent Torbjörn Edlund, Dr. Hans Ericsson, Dr. Bo Hallgren, Docent Per Lundström, and Dr. Bengt Persson, for valuable help and criticism.

I wish to thank Mr. Erik Askelöf of Pharmacia Ltd., and his scientific collaborators, for help and support.

To Professor Bernhard Jacobowsky I am very grateful for the privilege of performing one part of the spectrophotometric measurements in his laboratory. For clinical material necessary for the investigation I am indebted to Professor John Naeslund, Dr. Gösta Öberg, and Dr. Anna-Lisa Annell.

The statistical calculations were carried out by Aktuarie Elvir Lander and for this valuable assistance I proffer him my appreciation.

I am especially grateful to my laboratory technician Miss Franzi Wagenberg, for her skilful, unsparing, and thorough work throughout the course of these investigations. I am also indebted to the whole staff of the Paediatric Clinic as well as the nursing staff of the labour ward.

Dr. Marcia Skogh revised my original manuscript. I wish to offer my thanks for her accurate and painstaking work.

Financial support has been gratefully received from the Medical Faculty of the University of Uppsala, from the Regnell Fund, and from the Scientific Foundation of Pharmacia Ltd.

Acta Pædiatrica **40**: 519—539. Nov. 1951.

FROM THE PEDIATRIC DEPARTMENT OF AKADEMISKA SJUKHUSET, UPPSALA. (HEAD: PROFESSOR BO VAHLQUIST.)

Intravenous Iron Therapy in Pediatrics

by

BENGT HAGBERG

Searches have been made for a very long time for compounds of iron suitable for intravenous injection. The earlier preparations which we know of have more disadvantages than advantages. Ordinary, easily dissociable salt solutions of the type of ferro ammonium citrate, ferro ascorbate or ferri hydroxide give unpleasant toxic symptoms even in doses of about 10 mg Fe in adults, i. e., in amounts that are of little use therapeutically. GOETSCH, MOORE and MINNICH (1946) made experiments by giving colloidal ferri hydroxide in single massive doses which at times exceeded 1000 mg Fe. Its use, however, involved such severe side reactions that the authors concluded that "parenteral administration of iron is impracticable, dangerous and unnecessary as a therapeutic procedure."

In 1947, however, NISSIM reported good results in hypochromic anemia with another iron compound complex, a saccharated oxide of iron, which was tolerated remarkably well even in massive single doses. This iron compound, in improved variations, has since been found to fulfill the requirements necessary for an injectable iron preparation better than all hitherto used. A good series of cases treated has now been published (1, 2, 3, 7, 10, 11, 14, 15, 16, 22, 24, 28). It would seem, however, that this form of therapy has been very little used in children. At all events one only comes across a few reports about this in the literature. Nevertheless, DICKSTEIN and others (1951) give a report concerning a large material comprising 80 children between the

ages of 2 months and 6 years suffering from iron deficiency anemia of a primary nutritive genesis. As a rule the hematologic responses were quick and good. The final hemoglobin level was usually determined within three weeks, and it averaged 12.2 g %. Slight toxic reactions were observed in only 3 out of 199 injections, despite the fact that the amount of iron per kg body weight seems to have been rather considerable.

In this paper, the author would like 1. to give an idea about the behaviour of saccharated oxide of iron in the blood, 2. to give an account of his clinical experiences in treating 21 children of whom the majority were suffering from anemia of iron deficiency type, and 3. to establish indications for the use of intravenous iron therapy in children.

1. The behaviour of saccharated oxide of iron in the blood

One cannot help wondering how it is that saccharated oxide of iron, which at times gives very high iron concentrations in serum, is for the most part so well tolerated. Experiences gained in recent years in connection with the way in which iron passes through the blood give valuable information on the matter (19). Iron that is not hemoglobin-bound, i. e. serum iron, with its normal value of approximately 100 γ-%, occurs bound to a special β_1-globulin, the transferrin or siderophillin, in a firm iron-protein complex. The total iron-binding capacity of normal serum amounts to 300—400 γ-%. Thus, only one third of the globulin fraction is used for iron-binding in ordinary cases. If one then injects an easily dissociable iron solution into the blood, the whole fraction is rapidly saturated with iron. According to the degree in which the capacity is exceeded and the surplus iron extravasated one gets the usual symptoms of iron intoxication (19). If, on the other hand, one injects saccharated oxide of iron, this appears initially in the blood in an unchanged form and a high concentration; besides there is partially saturation of the iron-binding globulin. Gradually, increasing amounts of soluble iron that can be added to this protein fraction are liberated (Fig. 1). The mechanism for the transformation of iron from

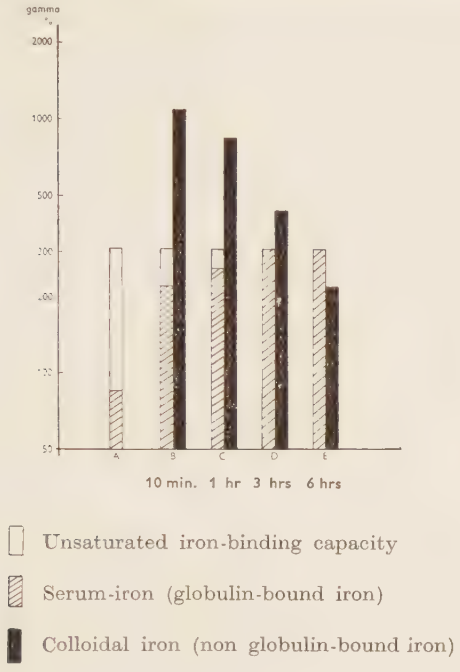


Fig. 1. The unsaturated iron-binding capacity and the iron content of serum before (A), and after intravenous injection of 100 mg Fe in 5 ml Intrafer (B—E). Note that the iron in saccharated iron preparations is not quantitatively regained with the ordinary serum-iron technique (30).
(The values are for a 22 year old nurse with a slight hypochromic anemia. She displayed no toxic reactions after the injection of Intrafer.)

a colloidal to an easy dissociable form is not quite clear. According to CAPPEL (1930) and ANDERSSON (1950) in experiments on mice and rabbits respectively, saccharated oxide of iron injected intravenously is taken up by reticulo-endothelial cells in the same way as suspensoid substances, e. g. carbon. In the reticulo-endothelial cells the iron is converted from a finely particulated to a soluble form and is redistributed to the depots, mainly the parenchymal cells of the liver. This process seems to be rather slow, taking days to weeks. It is the present author's opinion that, in addition, there may also be a breaking down of saccharated iron directly in the blood, perhaps due to an enzymatic

activity. Increasing saturation of the iron-binding capacity during the first few hours after the injection, speaks in favour of this theory (Fig. 1).

2. Therapeutic experiences

Material: The 21 cases treated (see Table 1) comprised 12 cases of iron deficiency anemia, one hemorrhagic anemia that had just recovered from a bout of rheumatic fever, four postinfectious anemic conditions of a slight nature, two cases of ulcerative colitis, one current nephritis, one case of lupus erythemathosus disseminatus with a constant feverish temperature, and lastly one case who refused to take food and who could not be persuaded to take iron per os. There were several types of iron deficiency anemias: a 12 year-old girl with a hereditary form; two cases of celiac disease, of which one was grave with a previous stubbornly resistant hypochromic anemia of many years standing; one case of ulcerative colitis that was in the process of healing and with secondary iron deficiency; three children of one year of age of the gruel-eating type with simple, nutritive iron deficiency; two other one year-old infants with hypochromic anemia following a prolonged condition of post infectious anorexia; and finally three idiots whose iron deficiency was of a mixed, though primarily nutritive nature.

The diagnosis of iron deficiency anemia was based on a decreased colour index, a low serum iron, and a raised iron-binding capacity in serum (the normal values in children are lacking for the time being but these, in view of the author's experiences, coincided to a large extent with those of adults). At least two, and in most cases all three criteria were manifest. Increase of the iron-binding capacity seemed to be consistent and most certain. This increase has been considered as being the more or less fruitless efforts of the organism to mobilize iron from the depots for the production of hemoglobin. An increase, then, of the unsaturated as compared with the saturated iron-binding globulin fraction is supposed to promote a greater flow of iron from the depots to the blood stream (19).

Methods: A spectrophotometric method in accordance with Rath-Finch (1949) was used in order to determine the unsaturated iron-binding capacity of the serum. The principle is based on the fact that a progressive development of red colour occurs on the addition of iron

to the β_1-globulin until the protein becomes saturated. The serum iron was determined in accordance with VAHLQUIST's method (1941) with minor modifications. The hemoglobin was measured with an *Autenrieth* hemoglobinometer. The usual clinical methods were used for examining the erythrocytes and reticulocytes.

Preparation, calculation and dosage: Various preparations of saccharated oxide of iron have appeared on the market. The author consistently used *Pharmacia*'s "Intrafer"[1] which is identical in manufacture with a preparation used chiefly in England called "Ferrivenin" marketed by BENGER. Each ampule of "Intrafer" contains 5 ml 2% saccharated oxide of iron corresponding to 100 mg molecular iron. The total amount required for each individual case can be conveniently calculated in accordance with the following formula: kg body wt. × Hb. deficiency in g% × 2.5 = mg Fe, where 2.5 is the blood volume factor. No regard, however, has been paid in the above formula to the impoverishment of iron deposits which usually must be taken into account with hypochromic anemia. In the following material the calculation therefore was estimated higher, namely:

$$\text{kg body wt.} \times (15 - \text{Hb. in g\%}) \times 2.5 = \text{mg Fe}$$

The method of administration for children originally suggested and which consisted of repeated doses (20 + 20 + 60 . . mg Fe) is difficult to carry out in practice in those ages most profusely represented in a series of pediatric iron deficiency anemias, namely those between one and two years. For this group especially, and even for somewhat older children, it is desirable that the number of injections should be reduced to the least possible, both for technical and psychological reasons. The author therefore tried to administer the preparation in the form of single massive doses which naturally would be the ideal method of administration.

Technique: The technical details in regard to the injections are of special interest to pediatricians. Amongst other things it is often impossible to keep to the slow rate of injection recommended. The author thinks, from his own experience, that this is of no significance. Despite the fact that, almost consistently, the injections were given rapidly to infants, it was only in one case that any direct outside symptoms were observed, and even these were very slight. The same thing applying to the speed of the injection applied to the recommended buffering with aspirated blood in the syringe. Further, it is stated that fewer side issues occur if glass syringes are used. As such syringes were used consistently, no opinion concerning the relevancy of this statement can be

[1] The iron preparation used was kindly supplied by Pharmacia Ltd., Manufacturing Chemists in Uppsala, Sweden.

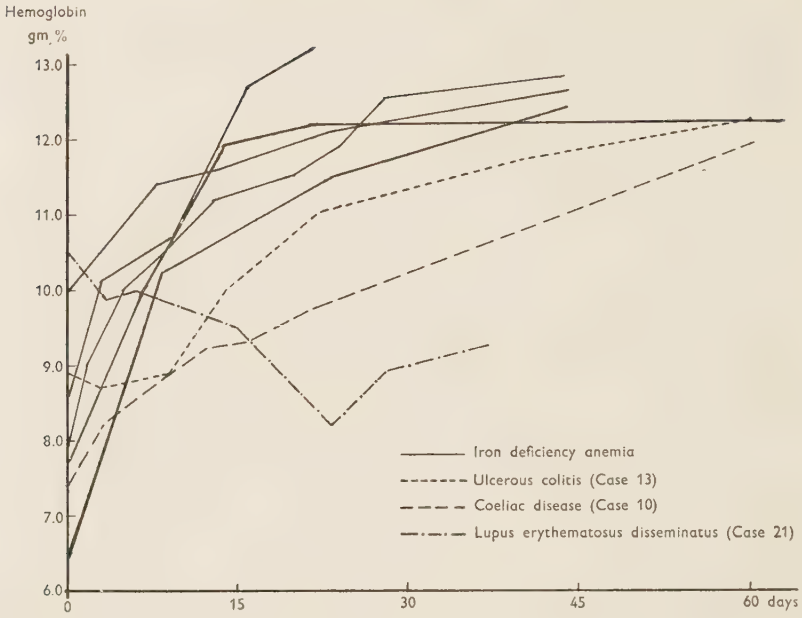


Fig. 2. Hemoglobin responses following intravenous iron therapy with saccharated oxide of iron (Intrafer).

given. On the other hand, the use of two different syringes, one for venipuncture and one for injection, can be recommended, as the brown-black, opaque colloid renders it difficult to verify the position of the puncture needle. It is true that the risks attending paravenous injections seem to be comparatively small, especially if the injection is made into a cubital vein, and yet one occasionally sees a necrosis such as, on one occasion, occurred on the dorsum of the foot.

Results and discussion: The results of treatment were consistently good in afebrile patients without current infection. In these the given dose entailed the estimated increase of hemoglobin (see Fig. 2). The majority reacted promptly with a surprisingly rapid increase of hemoglobin (cases 1, 2, 3, 5, 6, 7, 8, 9, 11 and 12). During the first two weeks there was an average daily increase of 0.25 g% in these cases. In differentiating from this type of hemoglobin response, one found in cases 4, 10 and 13 a slower remission curve which, after the first week, stayed

at an unchanged, or very slightly raised level. Decreased plasma protein values have been supposed to be one possible cause of the slow responses (24). This occurred in case 10, a boy with celiac disease, described in detail below. He had plasma protein values of about 6 g%. The maximum hemoglobin value, however, was nearly the same as that of the first group. On the whole, sideropenic children attained a hemoglobin value of 12.5 g% (12.2—13.7), which corresponds to a fully satisfactory normal value. The same high maximum hemoglobin value was obtained even in the remaining mixed cases, except for the last case, an 11 year-old girl with grave lupus erythematosus disseminatus and fever of a continuous type. Despite the fact that large doses of iron were administered repeatedly she showed a reduction rather than an increase of both hemoglobin and serum iron. With regard to the others, who all responded well, one must not forget that the spontaneous hemoglobin remission capacity is great after both infection and hemorrhage. For this reason there is no justification for any quantitative estimations in these mixed cases. Besides this, the infections in many of the cases were already totally or partially abated when iron was given. One detail which is not manifest in Fig. 2 is the slight initial decrease (0.3—0.7 g %) in hemoglobin values which was usually seen on the day following injection and which is possibly due to hemodilution (24, 17).

The changes in the red blood cell picture were insignificant in comparison with the marked increase of hemoglobin. Despite this, however, a rather typical reticulocyte crisis was observed in some of the cases. Such significant summits as have been registered in adults, 200 per thousand and over, have not been observed. As a rule one saw the maximum on the 3rd to the 7th day. Characteristically enough, there was no manifestation of any reticulocyte summit in two of the cases that had a slow hemoglobin remission, until very much later, namely on the 19th day in case No. 10, and on the 14th day in case No. 13.

An increase of the serum iron to normal values could be observed in the children with good hemoglobin remissions. The initial effect on the total iron content of the plasma was marked.

Table

Case no. sex age, yrs	Diagnosis	Before Treatment			
		γ % SI	γ % TIBC	Colour index	g % Hb
1. ♂ 1½	Iron Deficiency Anemia	40	490	0.77	7.7
2. ♂ 1	Iron Deficiency Anemia	54	534	0.60	8.7
3. ♂ 1	Iron Deficiency Anemia	46	493	1.00	10.4
4. ♀ 1	Iron Deficiency Anemia	72	452	0.72	10.0
5. ♀ 1	Iron Deficiency and Megaloblastic Anemia	64	501	0.65	8.0
6. ♀ 12	Iron Deficiency Anemia (essential hereditary.)	31	531	0.48	6.1
7. ♂ 5	Iron Deficiency Anemia + Idiocy	78	518	0.74	7.5
8. ♂ 6	Iron Deficiency Anemia + Idiocy	80	515	0.73	10.0
9. ♂ 1	Iron Deficiency Anemia + Idiocy	40	490	0.83	8.6
10. ♂ 8	Celiac Disease + Iron Deficiency Anemia	62	—	0.69	7.9
11. ♂ 2	Celiac Disease + Iron Deficiency Anemia	28	478	0.84	10.0
12. ♀ 1	Ulcerative Colitis + Iron Deficiency Anemia	76	561	0.80	10.5
13. ♀ 5	Ulcerative Colitis + Iron Deficiency Anemia	54	379	0.85	8.9
14. ♀ 8	Anemia Sec. (Blood Loss)	22	302	1.03	9.2
15. ♀ 10	Anemia of Infection (Status post febr. rheum.)	96	276	0.92	10.2
16. ♀ 2	Anemia of Infection (Status post Bronchit.)	98	328	0.93	10.5
17. ♂ 5½	Anemia of Infection (Lymfadenitis colli sanans)	36	251	1.00	10.5
18. ♂ 4	Anemia of Infection (Status post pneumon.)	52	287	1.00	10.9
19. ♂ 5	Nephritis + Anemia	88	438	1.00	10.7
20. ♀ 3½	Anorexia	160	345	0.95	11.0
21. ♀ 11	Disseminated Lupus Erythemathosus	104	339	1.00	10.5

SI = Serum Iron.
TIBC = Total iron-binding capacity of serum.

1.

After Treatment					mg Fe (Intrafer)			Number of injection
1 w. g % Hb	2 w. g % Hb	3—4 w. g % Hb	2 mths g % Hb	Max. Retic-‰	Calculated need	Injected	Single doses per kg	
10.0	11.9	12.1	12.2	—	180	100	10	1
11.1	—	12.0	10.5	60 (2)[1]	150	110	11	1
11.9	11.7	12.2	—	—	110	50	5	1
10.3	—	11.9	12.0	—	100	90	11	1
10.5	11.2	12.5	13.0	62 (3)	160	180	11	2
8.4	—	10.4	[2]	88 (5)	800	500	7	3
10.2	—	11.5	12.4	—	375	300	15	1
11.4	—	12.1	12.6	—	225	220	11	1
10.7	12.7	13.2	mors	95 (4)	120	250	17	3
8.9	9.2	9.7	11.7	130 (19)	350	200	10	1
11.7	11.7	12.2	10.7	54 (3)	150	100	9	1
12.7	—	13.7	—	23 (4)	120	90	10	1
8.9	10.0	11.0	12.2	58 (14)	260	200	6	2
—	11.0	12.5	12.7	—	400	200	3.5	2
11.5	12.1	12.7	12.0	28 (7)	300	300	4	3
12.0	11.4	—	12.3	23 (6)	100	50	6	1
12.6	12.6	—	—	37 (7)	250	250	13	1
12.7	12.0	13.5	—	—	115	150	9	1
11.2	11.7	12.3	12.5	—	225	100	5	1
12.2	12.2	—	—	11 (4)	60	60	5	1
10.0	9.5	8.8	8.2	35 (4)	315	400	7	3

[1] = Day for the maximal reticulocyte count.
[2] = Peroral iron therapy added.

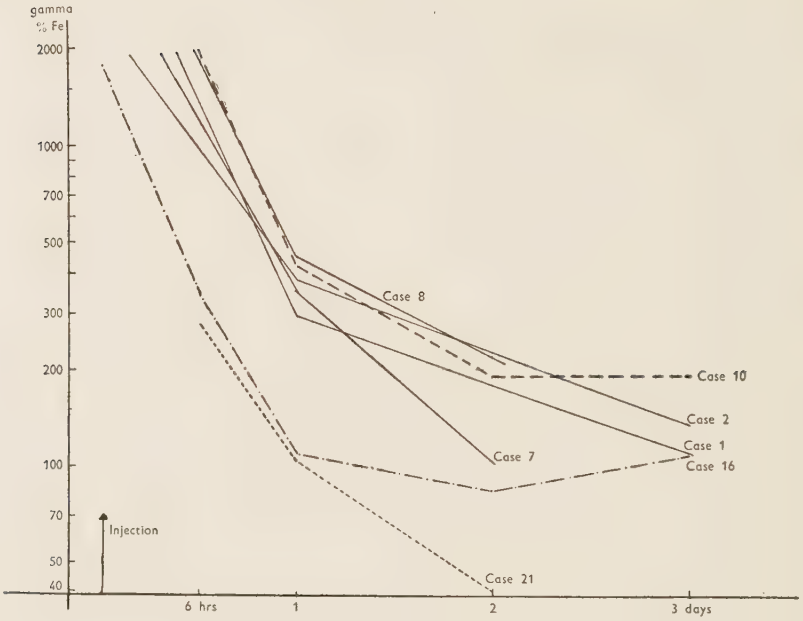


Fig. 3. The immediate effect of i. v. saccharated oxide of iron on the iron content in serum.

Immediately after the injection (i. e. 10 minutes), iron concentrations of up to 6000 γ% were measured (case 2). And even after a lapse of 6 hours the concentrations were, as a rule, still high, about 1000 γ%. After one day they fell to between 200 and 400 γ% (see Fig. 3). The iron-binding capacity was continuously followed in one single case that remained for a long time in hospital. As will be seen from table 2, case No. 11 (celiac disease) manifested a definite reduction during the month following the treatment. Despite a satisfactory hemoglobin value and a normal serum iron, the iron-binding capacity, however, did not fall below 400 γ%, probably emphasizing a latent deposit deficiency of iron. From table 1 it also appears that only two thirds of the estimated need of iron was given in the single, massive injection and that the therapeutic effect did not last for the two months of control.

Some of the cases have their own special interest because they

Days in relat. to injection	γ % SI	γ % TIBC	g % Hb
— 1	28	478	10.0
+ 6	96	—	11.7
+ 21	102	452	12.2
+ 33	90	425	12.2

Table 2.

Case 11. 2 year-old boy with celiac disease and slight iron deficiency anemia. The changes in serum-iron (SI) and total iron-binding capacity (TIBC) during the month following one single injection of 5 ml Intrafer (= 100 mg Fe). The values of the TIBC give a more adequate knowledge of a latent iron deficiency.

previously proved to be resistant to treatment by repeated and lengthy peroral iron therapy. A report of two such cases, each representing one of the two types of hemoglobin response will now be given. The first of these also showed the most noticeable toxic symptoms in the material.

Case No. 5.

A 15-month-old girl who weighed 1.600 g at birth. She was nursed in the premature ward and, after little more than a month, developed anemia which, in the beginning, was normochromic and then became hypochromic with a hemoglobin value of about 8 to 9 g%. In spite of intensive iron therapy per os which was carried out for practically the whole of her first year of life, there was no improvement. At times she reacted by vomiting, for which reason different iron preparations were used. For about two months before the actual treatment she had been getting 20 drops of "Guttafer" twice a day (= 70 mg Fe per diem). She was admitted here for parenteral iron therapy. Her weight was 9.000 g; she was small, delicate and pale. No epithelial symptoms. Otherwise there was nothing of interest in the status. The liver and spleen were not palpable. The blood values appear in table 1. Differential count showed a preponderance of lymphocytes but no immature cells. Erythrocytes presented a strongly pronounced anisocytosis and poikilocytosis. Price-Jones curve was broad with an average diameter of 6.6 μ. Tibial puncture revealed intensified erythropoiesis with numerous immature red cells which were partly megaloblastic in character. The erythrocytes presented a pronounced anisocytosis with rather many large megalocytic elements.

The girl was given two injections of "Intrafer", the first one being 4 ml (= 80 mg Fe), and the second 5 ml (= 100 mg Fe). On the first occasion her temperature rose to 38°.5 C on the same afternoon. Otherwise there was nothing of note. The second injection 10 days later brought on intense shivering after 3/4 of an hour, peripheral cyanosis and cold hands and feet, cold sweat and a pronounced general pallor similar to that seen in the beginning of a state of collapse. Notwithstanding, she was capable of screaming during the whole of this time and her general condition was never dangerous. She was given a stimulant of 0.4 ml Nicetamid. Half an hour later her colour improved and the extremities were warm. A couple of hours later she appeared quite unaffected, but had a temperature of 40°.5 C. The following morning there was nothing of note and she was afebrile. Hemoglobin remissions were good and rapid with a moderate reticulocyte summit. Diminished anisocytosis in the peripheral blood. A new tibial puncture showed, on the whole, a more mature erythropoiesis with only one or two cells that were megaloblastic in character. For this reason a total of 35 mg vitamin B_{12} was given a good three weeks after the first injection of "Intrafer". Nevertheless a complete normalization of the bone marrow was not achieved during the following week. She was discharged from the hospital in an extraordinarily improved condition three weeks after the beginning of the treatment and had a hemoglobin value of 12.5 g% and 5.05 million red blood corpuscles. One month later a control examination was made and she was found to be in the same good state of health, with a hemoglobin value of 13.0 g%.

Case No. 10.

A 9 year-old boy with an early beginning and exceptionally severe form of celiac disease. He had had grave and threatening celiacal crises, repeated states of tetany, negative balance of fat metabolism, muscular atrophies, skeletal changes together with typical psychic alterations. At the age of nine, he showed a physical development corresponding to a child of from 4 to 5 years of age. His body weight was 19 kg. Right from his early years he had had hypochromic anemia which, in the beginning, in any case, responded to oral iron therapy. At the age of six the boy was again admitted with a hemoglobin value of about 9 to 10 g%. On this occasion, and ever since, he no longer reacted well to iron per os, but instead was afflicted with increased gastro-intestinal trouble. For this reason treatment for anemia was avoided. During the half year prior to intravenous iron therapy, the anemia was markedly hypochromic with a colour index of between 0.6 and 0.7, and a hemoglobin value of between 7.5 and 9.5 g%. The red blood cell picture showed pronounced anisocytosis and poikilocytosis with a corpuscular diameter varying between 5.5 and 10.0 μ. The average diameter was 7.27 μ. The

reticulocytes were constantly about 30 to 40 per thousand. No bone marrow punctures were made due to the patient's violent psychic reactions to physical pain. Plasma proteins were low; during the last half year between 5 and 6 g %. The decrease primarily concerned the albumin, but nevertheless the albumin/globulin quotient was usually above 1.0. Prior to the "Intrafer" treatment he got, as a trial for two months, massive doses of B_{12}, in all 160 microgrammes. During the latter part of the time of treatment this was combined with oral iron therapy, all without having the slightest effect on the low blood values. On a previous occasion, folic acid had been tried as well, but with the same negative results. It was therefore decided to give iron intravenously. On account of the atypical way in which the patient reacted, as well as because of his sensitive constitution, the administration was carried out by means of intravenous drip; 10 ml "Intrafer" (= 200 mg Fe) was given in 350 cc 5 % glucose during the course of 4 ½ hours. No toxic reactions were noted. During the following two weeks there was only a slow increase of hemoglobin, and no increased reticulocytosis. Thereafter, there was a rapid increase of the reticulocytes which reached the maximum of 130 per thousand on the 19th day. There was still the same slow, but progressive increase of the hemoglobin content. During the follow-up examination two months after the treatment, the patient was in a surprisingly good condition. The hemoglobin value had increased to 11.9 g %, and the colour index has risen to 0.88. These values must be regarded as satisfactory, especially as he was given considerably smaller doses than the estimated need. Barely two weeks later, however, the boy entered into a new period of decline accompanied by edema, tetany, and a generally low state of health. After recovering from this, he again showed a fall in the blood values. Nevertheless, the hemoglobin now lay on a higher level than the year previously, viz. 10—11 g %, whereas the index remained at about 0.8.

Toxic effects: The unpleasant, incipient shock picture related above, fortunately seems to be rare, at all events if one keeps within moderate injection doses. From table 3 it appears that single massive doses can nevertheless be tolerated well by certain children, but that the risk seems to be considerably greater if one exceeds a dose of about 10 mg per kg body weight. In all probability, symptoms can even arise in individual cases with much smaller amounts, but under these circumstances the risk of a real state of shock ought to be minimal. It is believeable that there is some connection between the tolerance capacity and the size of the iron-binding capacity. It should therefore

Table 3.

Toxic manifestations after intravenous injections of saccharated oxide of iron (Intrafer) in single doses.

Mg Fe injected per kg body wt.	Number of injections	Local reactions (thrombophlebitis)	On injection slight toxic manifestations Flushing Cough Tiredness	1—4 hrs Moderate toxic manifestations Nausea Paleness Shivering Temp. > 39° C	¾ hr Strong toxic manifestations Incipient shock Pale cyanosis Shivering Temp. > 40° C.	Vasovagal Shock
0— 5	6	0	0	0	0	0
5—10	20	1	1	0	0	0
10—20	8	0	0	3	1	0

be emphasized that large single massive doses have, in these cases, mostly been given to children with sideropenia, that is to say with a considerably raised value for the iron-binding capacity. Another possibility is that the growing organism may be incapable of breaking down the saccharated oxide of iron complex as quickly as some adults. However, very little, if anything at all, is known about the mechanism of the intoxication symptoms. Under such conditions, one may well ask if it is necessary to give single massive doses as has been done in this case. Naturally, it is not necessary, but for technical and psychological reasons it is especially important to avoid giving irritating, daily doses, at all events, to the smaller children. In cases where one has reason to be especially careful, it would be preferable to resort to an intravenous drip using a 5 % glucose vehicle as the dilution medium.

It would appear that toxic symptoms have a tendency to make their appearance between one and four hours after the injection. There was only one case that showed any sort of reaction in connection with the actual injection, and only an extremely mild one at that. This was a girl of 1 year of age, who had a general flushing of the skin and a slight irritable cough. The manifestations that appeared later began with pallor, a cold sweat and a rather severe attack of shivering (cases 4, 5 and 17), followed by a rapid transitory high temperature of about 40°C. Of these three cases it was only Case 5, as previously reported,

who manifested an alarming circulatory insufficiency. In all three, however, the symptoms subsided during the course of an hour or so. Over and above these palpable general symptoms it is to be noted that a boy suffering from a troublesome nephritis (case 19) showed an increased hematuria — though only during the day following a moderately large single injection. Notwithstanding, there were a number of other cases that had been given considerably larger single massive doses who showed normal sediments many hours and days after the injections. No eosinophilia in connection with single or repeated doses has been observed.

The other aspect of the intoxication problem, i. e. any eventual late manifestations in connection with too large a total dose of iron, has not yet been fully investigated. Only a few autopsies of cases treated with saccharated oxide of iron have, so far, been published. The lapse of time after the last injection has in no case been more than a couple of months. Hitherto, none of the cases have shown any beginning fibrosis or other damage to the parenchymal organs. Experiences with experimental animals speaks in favour of pure iron preparations as relatively inert, even in large doses. Brown, Moore and others (1950) have given dogs up to 1,000 mg iron per kg body weight without any signs of cirrhosis 15 months after the treatment. Andersson (1950) came to the same conclusions in his observations of 110 rabbits. Thus it seems reasonable to think that iron, while important, is probably not the only etiologic factor in producing the symptomatic form of hemochromatosis, such as occurs after repeated transfusions.

One case in the author's material died from an intercurrent disease six weeks following a vigorous and fully completed treatment. The results of the autopsy are of special interest as exceptionally massive doses of saccharated iron had been given.

Case No. 9.

A boy, aged 1 year and 2 months, weighing 7 kg; scaphocephalic and grossly underdeveloped. He was previously a patient in the Children's Department on account of transient but recidivating cystopyelitis. Hypochromic anemia noted half a year previously. Hematologically examined

while in an uninfected state one week after his last short pyelitic incident, and treated with large doses of "Intrafer" as a considerable iron deficiency was observed (see Table 1). Rapid powerful hemoglobin remissions. Sent home, in a decidedly improved state of health all things considered. He remained so for the next six weeks but died suddenly from an acute hyperpyretic climax which, in all probability, was a relapse of cystopyelitis.

Autopsy diagnosis (Dr. Ingrid Lingmark): Pyelonephritis chronica + Bronchitis acuta + Bronchopneumoniae.

Histologic examination showed a profusion of hemosiderin in the reticuloendothelial cells in the spleen, only a moderate amount in the Kupffer cells and no damage to the parenchymal cells of the liver (see Fig. 4). Negative iron staining of preparations from the kidneys, lungs and pancreas. Bone marrow, nothing of note.

Quantitative chemical determination (Dr. Bo Hallgren): See Table 4.

Up to the present, then, no proof has been found of pathologic parenchymal changes even from very vigorous intravenous iron therapy. On the other hand, the longest time of observation has been little more than one year, even in respect to experiments carried out on animals. It is therefore necessary to exercise care in regard to the total amount of iron injected. Possibly, the risks have been exaggerated.

3. Indications for the use of saccharated oxide of iron in children

Since the response of patients with iron deficiency anemia to oral iron therapy usually is good, there are few real indications for giving iron intravenously. For practical and psychological reasons, this especially applies to children. Under certain conditions, however, one may talk about definite indications, namely in iron-deficient children who

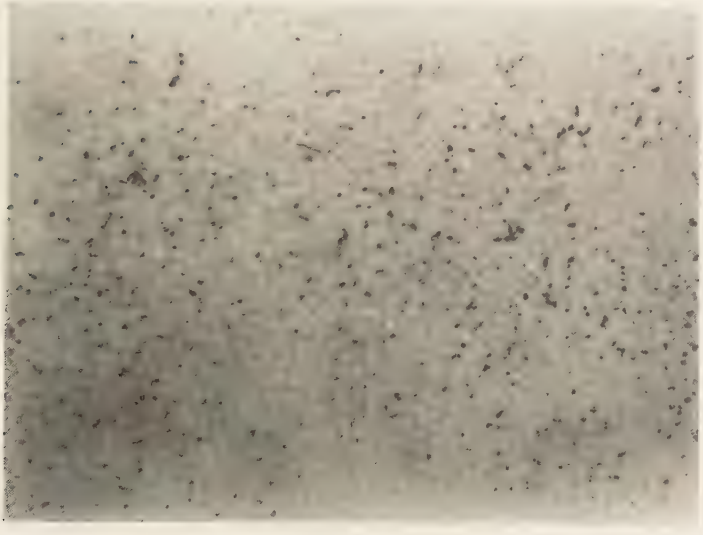
1. are unable to derive full benefit from iron administered orally as for instance in celiac disease.

2. cannot tolerate oral iron therapy without nausea, diarrhea, etc.

3. must have their intestines shielded from every conceiveable mucosal irritant, e. g. in ulcerative colitis.

Sometimes it may also be considered advisable to give an initial single massive dose of iron intravenously in cases of severe

A



B

Fig. 4. *Case 9.* Iron granules in spleen (*A*) and liver (*B*).

Table 4.

Case 9. Quantitative chemical determinations of hydrolyzable iron in some organs. (The values received from Dr Bo Hallgren, Institute of Medical Chemistry, Uppsala, who performed these analyses.)

Organ	Weight in g	Mg Fe per 100 g organ (fresh weight)	Total amount Fe in mg
Liver	207	40	82.8
Spleen	13	99	12.9
Kidneys ...	26	3.4	0.9
Lungs	148	2.0	3.0
Heart	34	1.9	0.7

but uncomplicated iron deficiency anemia of the nutritive type. By doing so, one may achieve an improvement in the patient's general condition and the hemoglobin value more quickly than otherwise.

There is no real reason to anticipate any hematological improvement in cases of anemia during the actual course of infection. SINCLAIR and DUTHIE (1949), however, have achieved remarkably good results in adults suffering from long standing rheumathoid arthritis with hypochromic anemia, resistant to previous oral iron therapy. There are, however, contradictory investigations on anemias of infection from other sources (18), and so, for the time being, this indication should be accepted with reservation.

In summary it may be said:

that saccharated oxide of iron is a valuable therapeutic in pediatrics within a limited range of indications,

that the hemoglobin remissions after treatment with saccharated oxide of iron in iron-deficient children are marked and rapid,

that children usually seem to tolerate proportionally large single doses of saccharated oxide of iron (Intrafer) quite well, up to about 10 mg per kg body weight,

that children up to the age of 2—3 years can, for practical and psychological reasons, be beneficially treated by giving single injections, not exceeding 10 mg per kg body weight,

that older children are more satisfactorily treated by repeated and somewhat smaller doses such as, for example, 5 mg per kg body weight at a time,

that sensitive cases, as well as those in need of very large total amounts of iron, should be given saccharated oxide of iron by means of a slow intravenous drip with 5 % glucose.

Finally, that care should be taken in regard to the total amount of iron injected. One should therefore keep within the estimated need of iron until many years of observation have proved the harmlessness of very large iron depots, and the beneficial, long lasting effect on a previously sideropenic organism.

Summary

The behaviour of saccharated oxide of iron in the blood is discussed. In addition to uptake and conversion of the iron complex in the reticuloendothelial cells, an increasing saturation of the iron-binding capacity during the first few hours following intravenous injection may indicate a liberation of soluble iron directly in the blood. Therapeutic trials with a Swedish preparation of saccharated iron (Intrafer, *Pharmacia*) are reported. Twentyone children with anemia, mainly of iron deficiency type, were studied. The diagnosis of iron deficiency was substantiated by a decreased colour index, a low serum iron and an increased iron-binding capacity of the serum. In all cases free from infection good hemoglobin responses were obtained. Among these were two cases who had not responded to long periods of oral iron treatment. The preparation was well tolerated in amazingly large single doses. Thus, repeated venipunctures often could be avoided. A few toxic reactions after massive doses (more than 10 mg Fe per kg body weight) were observed. The question of possible late toxic manifestations on the parenchymal organs is discussed. The indications for the use of intravenous iron in childhood are set forth and a scheme for the calculation of the approximate need of iron is given.

La thérapeutique par le fer intraveineux en pédiatrie.

L'auteur traite de la destinée dans le sang d'un oxysaccharide de fer. Outre un dépôt et une transformation d'un complexe ferreux dans le système réticuloendothélial, on peut observer, pendant les premières heures qui suivent une injection intraveineuse, une saturation croissante de la capacité de fixation du fer par libération de fer directement dans le sang. On rapporte une expérience thérapeutique faite avec un sac-

charide de fer, préparé en Suède (Intrafer, Pharmacia). Vingt et un enfants, avec anémie, essentiellement de type ferriprive, ont été observés. Le diagnostic de carence en fer repose sur une diminution de la valeur globulaire, un taux bas de fer dans le sérum et une capacité croissante de fixation de fer. Dans tous les cas sans infection, de bons taux d'hémoglobine ont été atteints. Parmi ceux-ci, se trouvaient deux cas qui n'avaient pas réagi à un traitement per os de longue durée. De façon surprenante, de grosses doses isolées de la préparation ont été bien supportées. Ainsi, des ponctions veineuses répétées ont pu être évitées. Après de grosses doses (de plus de 10 mg de Fe par kilo) quelques signes toxiques ont été observés. La possibilité d'une action toxique tardive sur les organes parenchymateux est discutée. Les indications de l'emploi du fer intraveineux chez l'enfant sont données, ainsi qu'un schéma pour le calcul des besoins approximatifs.

Die intravenöse Eisentherapie in der Kinderheilkunde.

Es wird das Verhalten von Eisenoxydsaccharat im Blute besprochen. Neben einer Aufnahme und Verwandlung des Eisenkomplexes in den retikulo-endothelialen Zellen kann eine zunehmende Sättigung der Eisenbindungsfähigkeit während der ersten Stunden nach intravenöser Injektion auf eine Dissoziation von löslichen Eisen direkt im Blut hinweisen. Es wird über therapeutische Versuche mit Anwendung eines schwedischen Eisensaccharatpräparates (Intrafer, Pharmacia) berichtet. Einundzwanzig Kinder, mit Anämie, hauptsächlich vom Eisenmangeltypus, wurden beobachtet. Der Eisenmangel wurde durch verminderten Farbenindex, niedrige Eisenwerte und eine gesteigerte Eisenbindungsfähigkeit des Serums diagnostiziert. In allen infektionsfreien Fällen wurden gute Hb-Werte erreicht. Darunter befanden sich zwei Fälle, bei welchen eine langdauernde perorale Behandlung nicht gewirkt hatte. Erstaunlich grosse Einzelgaben des Präparates wurden gut vertragen. Auf diese Weise konnten wiederholte Venenpunktionen vermieden werden. Nach grossen Gaben (von mehr als 10 mg Fe pro kg Körpergewicht) wurden einige toxische Auswirkungen beobachtet. Es wird die Möglichkeit einer späteren toxischen Auswirkung auf die parenchymatösen Organe besprochen.

Es wird die Indikation für den intravenösen Eisengebrauch im Kindesalter angegeben und ein Schema zur Berechnung des annähernden Bedürfnisses an Eisen mitgeteilt.

Terapéutica con hierro intravenoso en pediatría.

Se discute la acción del óxido de hierro sacaratado en la sangre. Al lado de un aumento y conversión del complejo férrico en las células reticuloendoteliales, un aumento de la saturación de los depósitos férricos

durante las primeras horas siguientes a la inyeción intravenosa puede indicar la liberación de hierro soluble directamente en la sangre. Se comunican los efectos obtenidos con la administración de un preparado de hierro sacaratado sueco (intrafer Pharmacia). 21 niños afectos de anemia primordialmente de tipo ferropénico han sido estudiados. El diagnóstico de deficiencia de hierro se comprobó por una disminución del valor globular, hierro sérico bajo. En todos los casos, excluidas por otra parte las infecciones asociadas, los valores de respuesta de la hemoglobina fueron buenos. Entre estos casos hubo 2 que no habían respuesto a la administración durante largos períodos de hierro por via oral. Los preparados son tolerados de un modo sorprendente a dosis amplias aisladas, por otra parte las punciones venosas repetidas frecuentemente deben evitarse. Se observó un escaso número de reacciones tóxicas tras la administración de dosis masivas (mas de 10 mg. de hierro por kilo de peso corporal). Se discute la cuestión de las posibles manifestaciones tóxicas tardías sobre los órganos parenquimatosos. Se sintetizan las indicaciones de uso de la terapeutica intravenosa con hierro en la infancia, y se aporta un esquema para el cálculo aproximado de las necesidades en hierro.

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Received 29.8 1951.

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The Iron-binding Capacity of Serum in Different Forms of Iron Poisoning

by

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Problems connected with toxic reactions following too high single doses of iron have come more and more to the fore in recent years. This is due in part to the following two factors. Firstly, colloidal, saccharated iron oxide, which can be given in huge doses, has revolutionized intravenous iron therapy and given it a wide-spread use. Secondly, iron medicaments are often supplied in the form of palatable dragees, much like the chocolate dragees of the confectioner. It is no wonder that children eagerly gobble them up.

The widened knowledge of the metabolism and transport of iron has opened up new lines of attack on problems concerning the mechanism of iron intoxication. Not least is the knowledge about the two closely related iron proteins ferritin (19, 12, 13) and transferrin (16, 30), which together are responsible for the iron transfer within the organism. While ferritin constitutes the mobile storage form of excess iron in the cells, transferrin (i. e. siderophilin, beta$_1$-metal-combining globulin) is the transport form of iron in the blood plasma. Facts so far known suggest that transferrin transports iron in the same way as haemoglobin transports oxygen, viz. without itself being metabolized (21). The protein fraction amounts to about 0.25 g/100 cc. Each transferrin molecule is capable of combining with two molecules of iron. The serum iron normally amounts to $^1/_3$ of the total iron-binding capacity (TIBC) of serum, which in the adult amounts to 300—360 gamma per cent iron (20, 28, 5). If the TIBC is exceeded symptoms of iron poisoning immediately appear.

Can this overloading of the TIBC explain all toxic symptoms and signs following the administration of iron in toxic doses? For the side-reactions following intravenous injections of highly soluble iron salts

such an explanation seems to be indisputable. In overdosage of iron by mouth there are two factors to be considered, firstly an often considerable local corrosion of the intestinal mucosa, and secondly a general iron poisoning (35, 39). The type of intoxication following intravenous injection of saccharated iron oxide is more complicated. Klopper (1951) states that 'toxic effects only arise when the iron-binding capacity is exceeded in the individual injection'. In studying the unsaturated iron-binding capacity (UIBC) after injections of colloidal iron, Cameron (1951) has arrived at the same opinion. Other authors have pointed out the clinical resemblance to reticulo-endothelial reactions (27). Gabrieli (1953), moreover, claims to have demonstrated toxic effects in the reticulo-endothelial system (RES) in animals following massive doses of saccharated iron oxide. Other explanations have also been put forward. Thus Schwietzer (1952) maintains that the collapse-reactions observed have probably been due to the formation of ferric hydroxide thrombi with secondary mechanical occlusion of various capillary systems, in particular that of the lungs. His assumption is supported by the findings of Nissim & Robson (1949) and Stille (1952) in animal experiments.

An account is given below of studies on the iron-binding capacity of serum in various forms of iron administration and iron poisoning.

MATERIAL AND METHODS

The investigations have mostly been carried out on nurses and student nurses. The iron tablets used were 'Novofer' and 'Novofer Forte' (Pharmacia)[1], containing 0.25 g and 0.45 g ferrous tartrate respectively (= 56 and 100 mg Fe), and 0.0025 g cupric sulphate. The preparations injected consisted of a solution of iron sulphate containing 0.04 g ferrous sulphate/ml (= 8 mg Fe), and a Swedish preparation of saccharated iron oxide, 'Intrafer' (Pharmacia), which is identical with 'Ferrivenin' (Benger). The preparation contains 20 mg Fe/ml. The serum iron (SI) has been estimated by the method of Vahlquist (1941), and the unsaturated iron-binding capacity of serum (UIBC) according to Rath & Finch (1949). The total iron-binding capacity (TIBC) is obtained by adding the values for SI and UIBC. Further details of the author's application of these methods are found elsewhere (15).

1. Oral administration of iron

The commonest symptoms of poisoning following overdosage of iron by mouth are given in table I. Several cases, almost all of them children, have been reported (6, 7, 26, 29, 33, 35). Spencer (1951) was able to demonstrate in two children a serum iron content exceeding 3 000 gamma per cent, viz. about ten times the TIBC. It is not known if the saturation limit is also exceeded in iron therapy accompanied by slight to moderate side-reactions. Laurell (1947) writes in connection

[1] The iron preparations used were kindly supplied by Pharmacia Ltd, Manufacturing Chemists in Uppsala, Sweden.

Table I.

	Oral Iron (poisoning)	Intravenous Iron (ferrous sulphate)	Intravenous Iron (saccharated oxide)
Immediate Reactions	None	Flushing Coughing Sneezing 'Pins and needles' Tachycardia Nausea Opression Slight shock	Flushing Coughing Transient leucopaenia Venous cramps Venous thrombosis
Delayed Reactions	*1—40 hours* Drowsiness Nausea Vomiting (haematemesis) Diarrhoea (melaena) Grey cyanosis Encephalopathy Hepatic failure (diffuse bleeding, icterus) Collapse Coma Death	None	*1—5 hours* Headache Aching of muscles High temperature Chills Nausea Vomiting Transient fall in blood pressure Shock

Toxic symptoms with different forms of iron administration.

with his oral tolerance tests: 'Ingestion of iron in large doses (up to 1 g) is often followed by nausea and diarrhoea, the explanation of which may very well be that the iron-binding capacity of the mucosa and serum is overtaxed, resulting in an abnormally high concentration of free iron ions in the intestinal wall, so that the mucosa cells become strongly irritated.'

In 17 iron tolerance tests carried out orally and on a fasting stomach general symptoms were noted in 5 cases, all of them women with slight hypochromic anaemia. The symptoms consisted constantly of nausea, retching, blanching, sweating, and faintness. They came on one to three hours after the beginning of the test, and lasted as a rule two or three hours. Two of the patients developed diarrhoea later in the day (Table II, cases 2 and 3).

As can be seen in table II, two of the five cases showed a fully saturated iron-binding capacity. It is possible that the saturation limit was even exceeded in cases 1 and 2, judging by the serum iron value. A small, unsaturated fraction (UIBC) was demonstrated in two of the cases. In the fifth and last case the turbidity of the serum prevented

Table II.

Case N:o	Age Sex	mg Fe (Novofer)	Before			2 Hours			4 Hours		
			SI	UIBC	TIBC	SI	UIBC	TIBC	SI	UIBC	TIBC
1. B. O.	23 ♀	280	68	340	408	224	194	418	432	0	432
2. I. L.	21 ♀	560	108	312	420	330	129	459	490	0	490
3. M. L.	30 ♀	500	75	375	450	254	218	472	394	44	439
4. G. K.	22 ♀	500	60	440	500	177	294	471	400	49	449
5. H. M.	22 ♀	500	56	320	376	108	264	372	233	—	—

The saturation of the iron-binding capacity after oral iron tolerance tests in five women who developed nausea, blanching, cold sweat, and drowsiness, 1—3 hours after ingestion.

accurate photometric determination. The serum iron level was, however, considerably lower than the initial TIBC value, thus there was probably an unsaturated fraction in this case also, as the TIBC evidently remains constant even in great fluctuations of the serum iron (20).

Discussion. The only conclusion that can be drawn from these few cases is that even relatively pronounced general symptoms, which might well be interpreted as a flooding of the organism with ionized iron, can occur without saturation of the TIBC. Most probably they can be regarded as due to the local irritation of the bowel. In contrast, the severe iron poisonings with toxic collapse are best explained by a penetration of the damaged iron-barrier of the corroded intestinal mucosa, and a massive exceeding of the TIBC in the blood. This interpretation is compatible with the very high iron concentrations found in serum by Spencer.

On the other hand the toxic symptoms observed here, combined with an incompletely saturated TIBC, might also be interpreted as evidence that poisoning following oral administration of iron does not necessarily depend upon an excess of free iron ions in the blood stream. J. P. Smith (1951) recently suggested that the clinical signs might be explained by excessive release of ferritin into the circulation. If this theory should be correct it does not follow that even with very high serum iron values the TIBC has been exceeded, because ferritin appears to be comparable with colloidal iron preparations. These do not always give a saturated TIBC in spite of an iron-level far above its capacity, as in the cases described.

Judging from Forbes's investigations (7) on similar preparations it is improbable that the minute quantities of copper present in the preparations used here have anything to do with the appearance of the toxic signs. Concerning its transport in serum, Holmberg & Laurell (1948) have shown that copper *in vivo* does not combine with transferrin, as was previously thought, but with a special copper-binding component, caeruloplasmin.

2. Intravenous dialysable iron (injectable ferrous sulphate)

Some years before the discovery of the iron-binding beta$_1$-globulin in the serum Skouge (1939) and Waldenström (1944) had already noted that the iron content of serum could be raised only to a certain limit, more or less constant from case to case, on intravenous administration of 10 mg of an iron salt. In untreated pernicious anaemia in particular the limit observed was far below the one calculated. Later, Holmberg & Laurell (1945) were able to show that this 'braking effect' of Waldenström was identical with the saturation limit for the complex-binding of iron, viz. TIBC.

If the TIBC is exceeded unpleasant symptoms appear within a few minutes, usually making impossible doses above 10 mg of iron in easily dialysable form. If no more iron is given the discomfort abates after a couple of minutes. The symptoms have been explained by vascular reactions occurring when ionized iron dialyses out through the capillaries (20). Most authors consider that iron not bound to transferrin disappears immediately from the blood stream (5, 10). Gitlow & Beyers (1952) believe this mechanism to be so effective that 'the five minutes specimen of the ten milligram intravenous iron tolerance test' is in general a simpler and better method for determination of the TIBC than those commonly used. I am not convinced that this is always the case, after comparing the five-minute values obtained from different normal series. Thus it is surprising that values obtained on seemingly comparable populations, such as the results of Brendstrup (1950) and Tötterman (1949), differ so materially. Brendstrup's 294 (270—348) gamma per cent agrees with Laurell's corresponding value for the saturation limit (315 gamma per cent). Tötterman's series, who received 10 mg Fe in the form of ferric chloride, gave values as high as 401 (366—445). Gitlow & Beyers explain discrepancies of this type by variations in the technique of blood-sampling and blood-analysis. Holmberg & Laurell (1945) have, however, shown in a few intravenous tolerance tests that small quantities of non-complex-bound iron can be demonstrated up to half an hour after injection. In their cases the amount of dipyridyl-reacting iron was of the order of 40—50 gamma per cent. Animal experiments point in the same direction. Moore and

his associates (1939) gave dogs 1 mg Fe per kg body-weight in the form of different iron salts. They obtained serum iron values as high as 1 600 gamma per cent, i. e. in all probability far exceeding the TIBC. The return to the initial value was gradual. Three hours after injection the value was still 800—1 000 gamma per cent. Neander & Vahlquist (1949) obtained similar results using rabbits, with doses of 0.5—2.0 mg Fe per kg body-weight.

To investigate the importance of the size of the dose of iron in assessing the TIBC by means of iron tolerance tests, varying amounts of ferrous sulphate have been administered. The TIBC was determined on samples taken before injection, and compared with the serum iron 15 minutes after the injection was complete. The injections were given slowly, over a period of 5—10 minutes.

Table III shows that doses of 0.16 mg Fe or more per kg body-weight gave serum iron values 15 minutes after the completion of the injection which considerably exceeded the TIBC. All toxic symptoms had disappeared at least 10 minutes before the blood samples were taken, with the exception of case 5, who felt uncomfortable for half an hour. In cases 11—25, where the iron solution was slowly injected until very slight toxic symptoms appeared (flushing, sneezing, and slight tachycardia) and was then immediately discontinued, the serum iron 15 minutes after injection in general corresponded well with the TIBC as measured before the injection. Although slight toxic symptoms were noticed the saturation limit was not reached in three cases. In two of them (cases 11 and 18) a persistent UIBC could still be shown.

Discussion. From the results it appears that the serum iron value 15 minutes after the injection of 10 mg of iron in dialysable form cannot always be considered representative of the TIBC. In normal cases weighing 70 kg this is usual, but in others it is often far from so. As Cartwright & Wintrobe (1949) state, the figures correspond when 0.13—0.14 mg Fe per kg body-weight is given to healthy adults (i. e. roughly 10 mg/70 kg). Rather than proceed from fixed doses it would seem to be preferable to inject the iron very slowly until the patient develops obvious flushing. Even then one cannot be absolutely certain of reaching the saturation limit, as can be seen from the three above-named exceptions. Possibly the transferrin cannot always complex-bind all available free iron ions, even if there is a residual UIBC. Here, too, some of the iron may pass through the capillary walls in free form, giving rise to symptoms. On the other hand it is remarkable that iron in excess of the saturation limit can remain in the serum for more than 10 minutes after the disappearance of the symptoms. Laurell (1947) has shown that this pathological iron fraction is dialysable, reacts directly with phenantrolin, and is adsorbed onto aluminum hydroxide. He calls the fraction 'loosely bound'. This loosely bound iron, too, seems to

Table III. *Intravenous iron tolerance tests with ferrous sulphate.*

Case N:o	Age Sex	mg Fe injected	mg Fe per kg b.-wt.	Before injection			15 min after		Reaction
				SI	UIBC	TIBC	SI	UIBC	
1. P. P.	6 ♂	16.0	0.78	80	422	502	900	—	3
2. R. J.	5 ♂	4.8	0.31	78	427	505	540	—	1
3. L. G.	22 ♀	13.6	0.302	96	247	343	546	—	3
4. R. O.	22 ♀	16.0	0.266	84	243	327	498	—	3
5. K. E.	21 ♀	16.0	0.25	82	278	360	576	—	4
6. M. W.	22 ♀	12.0	0.222	126	242	368	432	—	3
7. E. L.	22 ♀	9.6	0.181	140	243	383	434	—	3
8. R. O.	22 ♀	9.6	0.16	138	213	351	486	—	2
9. R. O.	22 ♀	9.6	0.16	160	184	344	443	—	2
10. L. G.	22 ♀	7.2	0.16	118	237	355	458	—	1
11. E. G.	30 ♀	9.2	0.144	47	427	469	333	104	1
12. S. J.	23 ♀	8.0	0.143	80	215	295	332	0	2
13. A. W.	23 ♀	7.8	0.142	139	193	332	333	0	2
14. B. J.	24 ♀	8.8	0.142	184	118	302	324	0	2
15. I. J.	32 ♀	7.2	0.140	110	216	326	329	0	1
16. U. G.	22 ♀	7.0	0.140	59	223	282	285	0	2
17. M. W.	22 ♀	7.2	0.139	129	229	358	343	0	2
18. B. G.	26 ♀	8.0	0.138	145	164	309	254	61	2
19. L. H.	23 ♀	8.0	0.138	59	437	496	525	0	1
20. E. L.	22 ♀	7.2	0.136	139	175	314	360	—	1
21. I. B.	22 ♀	7.0	0.135	76	257	333	334	0	1
22. M. W.	22 ♀	7.2	0.134	92	266	358	336	—	1
23. H. Å.	21 ♀	7.2	0.133	88	246	334	315	0	2
24. E. F.	27 ♀	8.0	0.133	133	198	331	357	0	1
25. H. M.	22 ♀	7.2	0.131	44	262	306	315	0	1
26. M. S.	22 ♀	6.4	0.0914	110	291	401	414	—	2
27. M. S.	22 ♀	6.4	0.0914	100	308	408	354	—	1

become attached to plasma proteins. On electrophoresis the two iron fractions seem, however, to move with different protein components. Wallenius (1952) has shown, by means of combined paper-electrophoresis and auto-radiography, that radioactive iron which was added to serum in amounts less than the TIBC combines only with the beta$_1$-globulin. On the other hand Vahlquist's (1941) electrophoretic investigations on sera which he enriched *in vitro* with $FeSO_4$ to a level far above known values for the TIBC suggest that most of the loosely bound iron moves with the albumin. Exactly how this iron is attached to the protein is, however, not known. Dialysis (20) reveals that it is partly ionized *in vitro* at physiological pH. How easily it is dissociated in the body is however unknown. Perhaps it is the serum's final, feeble stand against a sudden, massive diffusion of free iron ions from the blood-stream.

3. Intravenous colloidal iron (saccharated iron oxide)

Complex-bound iron in the form of saccharated iron oxide is tolerated in doses 10—100 times higher than in the easily dialysed form. Although the iron content of the serum becomes much higher than the TIBC this is not always fully saturated (fig. 1 A). This suggests strongly that the saccharated iron oxide remains in the first place to a large extent unchanged in the blood. Cameron (1951) followed the UIBC after injection of saccharated iron oxide. In none of his 6 cases was there complete saturation, even though the UIBC was constantly lowered. All his cases tolerated saccharated iron oxide excellently. He drew the conclusions that insufficient 'free iron' was liberated, in the doses used by him, to exceed the TIBC. Nor did he encounter toxic reactions, although he expected these with higher doses. And this is often the case. Occasionally, however, the toxic reactions are met with more erratically, at times with remarkably low doses. This instigated the following investigation of the UIBC both after varying doses of iron, and in cases with and without toxic reactions.

As a measure of the effect of varying large doses of saccharated iron oxide the decrease in the UIBC (5—10 minutes after injection) was used and expressed as 'increase in saturation'. It can be seen from table IV that the saturation on the whole shows a tendency to increase in proportion to the dosage. The individual variations are great, and the average results give only the general trend. Variations between

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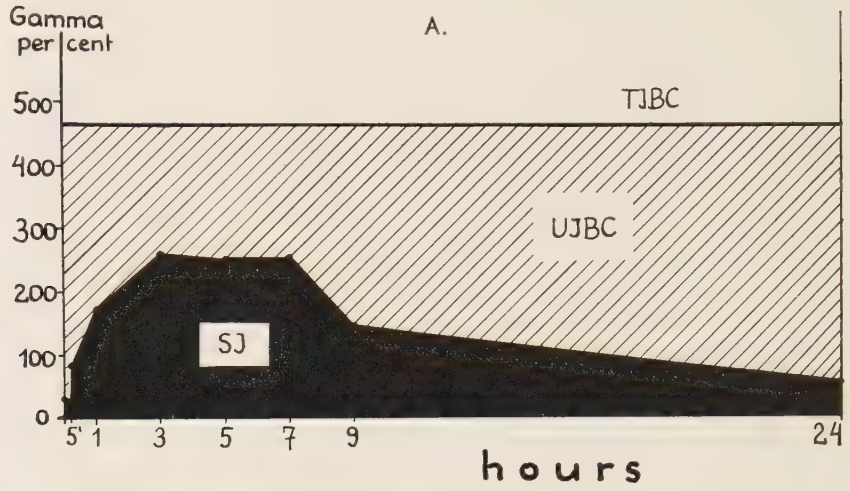
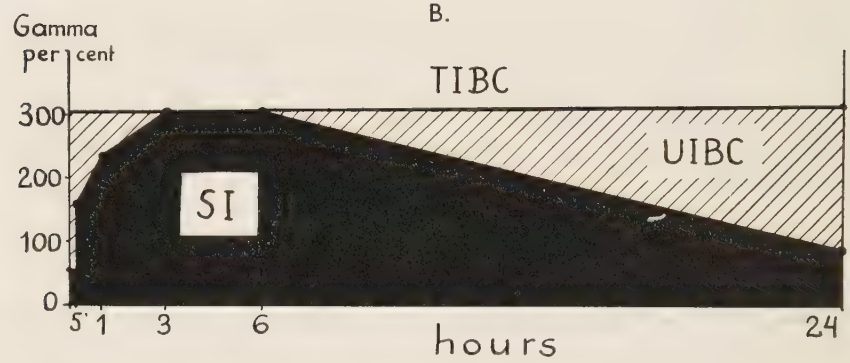
Intravenous iron tolerance tests with ferrous sulphate. The toxic reactions observed are divided into four groups. 1. Flushing. 2. Flushing, coughing, sneezing, 'pins and needles'. 3. In addition tachycardia, nausea, and oppression. 4. In addition slight shock.





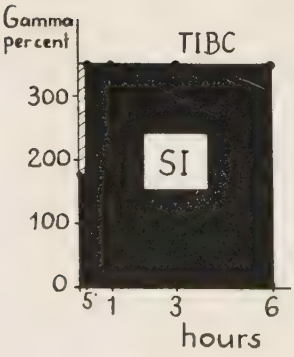


Figure 1.

The unsaturated iron-binding capacity (UIBC) in the hours following intravenous administration of saccharated iron oxide. The real serum iron concentration (SI) cannot be measured independently of the colloidal iron present, but has been calculated (TIBC before injection minus UIBC).

A. 27-year-old woman with chills, pyrexia, nausea, and headache, beginning five hours after 2.5 ml 'Intrafer' (50 mg Fe).

B. 21-year-old woman with no reaction after 5 ml 'Intrafer' (100 mg Fe).

C. 22-year-old woman with no reaction after 10 ml 'Intrafer' (200 mg Fe).

Table IV.

	Number of injections	Mg Fe Colloidal iron	TIBC before injection Gamma-%	Decrease of UIBC 5–10 min. after injection/adding Gamma-%	Increase in saturation (per cent of TIBC)
In vivo	11	50	441	60	14
	13	100	414	100	26
	10	200	391	237	60
In vitro	5	100[1]	346	120	35

The decrease of UIBC and 'increase in saturation' 5–10 min. after different doses of saccharated iron oxide.

different examinations in the same individual and with the same dose are also significant. Whether different batches of the preparation also give different values could therefore not be determined.

After 23 injections of 'Intrafer' (12 patients) the UIBC and SI were followed continuously over the next few hours. As many cases as possible which had toxic reactions were investigated, and hence the frequency of injections with side-effects is unproportionally high (5/24). The clinically-significant toxic reactions seen after 'Intrafer' and 'Ferrivenin' are mostly encountered 1—5 hours after injection. The clinical picture is given in table I. Really serious collapse seems to be extremely rare, and no fatal case has, to my knowledge, been described. The cases followed here had pyrexia, rigors, and vomiting. All but one can be described as moderate. The exception was a 25-year old woman who had received only 50 mg of iron. She developed, $3\,^3/_4$ hours after the injection, violent intermittent rigors, nausea, and paraesthesias, with extreme paleness, and cyanosis of the lips and nails. At times she appeared somewhat shocked. The symptoms lasted about 2 hours, but the patient had to remain in bed for the rest of the day. Bacteriological examination as well as animal tests (Pharmacia) of ampoules from the used batch of the preparation revealed no faults. Nor did pyrogen tests give any reaction.

A 43-year-old woman, one of the 'non-toxic' cases, developed another type of reaction. After each of 8 injections of between 100 and 300 mg of iron she reacted within a few minutes with sudden, transitory flushing, and a slight irritating cough. Her TIBC 5—10 minutes after injection was sometimes saturated, sometimes unsaturated. The symptoms were the same with varying doses, and their severity could not be related to the speed of injection.

[1] Approximately corresponding concentration.

Table V.

Injections of colloidal iron (Intrafer)	TIBC saturated during the first 3–6 hours	TIBC not saturated during the first 3–6 hours
Without toxic reactions	14 (20–200 mg Fe)	4 (50–100 mg Fe)
With toxic reactions	3 (50–160 mg Fe)	2 (50–100 mg Fe)

The saturation of the TIBC in relation to toxic reactions following injections of saccharated iron oxide.

The patients examined in the hours following the injection can be divided into four groups, with reference to the UIBC (table V).

1. Cases *not saturated* with iron up to the limit of capacity, and who were *symptom-free.*
2. Those *saturated,* sometimes rapidly, but nevertheless *symptom-free* (fig. 1 B & C).
3. Cases *saturated* and with *symptoms.*
4. One case who developed *definite symptoms* after two successive injections, but who remained, however, *unsaturated* (fig. 1 A).

Discussion. The mechanism by which iron is transformed from a colloidal to an easily dissociable form is not quite clear. According to Cappel (1930) and Andersson (1950), who experimented on mice and rabbits respectively, saccharated iron oxide injected intravenously is taken up by reticulo-endothelial cells in the same way as suspensoid substances, e. g. carbon. In the reticulo-endothelial cells the iron is converted from a finely-dispersed to a soluble form and is redistributed to the depots, mainly the parenchymal cells of the liver. This process seems to be slow, taking days or weeks. In addition to the reticulo-endothelial transformation, a direct splitting up of the colloid appears to take place in the blood (3, 14). The usual successively diminishing UIBC suggests this. It cannot be shown completely, however, as, with the methods used here, the TIBC remains unknown during the hours following injection of iron colloid, when the plasma iron concentration far exceeds the saturation limit (fig. 2). Thus it is not possible on the results of the tests to exclude that the reduction in the UIBC is caused, wholly or in part, by other factors. One possibility is that plasma protein is precipitated by the saccharated iron oxide, another that the photometric method does not give adequate results; the colour of the plasma undoubtedly is darkened somewhat by the black-brown colloid. Finally, a third possibility is that the TIBC diminishes physiologically with the great accumulation of iron in the organism.

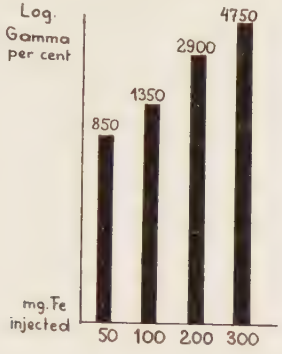


Figure 2.

The average iron content of serum 5—10 minutes after intravenous injections of different doses of saccharated iron oxide. N. B. the iron in preparations of this kind is not quantitatively recovered with the ordinary serum-iron technique (14 b).

Concerning the precipitation of the protein, it is known from the chemistry of colloids that positively-charged colloids precipitate negatively-charged. In the blood saccharated iron oxide behaves exactly like a positively-charged colloid (36). Stille (1952) has shown that precipitation of the negatively-charged plasma protein can indeed take place. He administered saccharated iron oxide intravenously to cats (100 mg/kg body-weight), and obtained vessel thrombi consisting of precipitated plasma protein and iron. Stille's doses were, however, more than 10 times those generally used in clinical practice. If precipitation of plasma protein is the explanation of the falling UIBC the symptoms would more likely have developed immediately following injection. Further, the UIBC should be at its lowest when the concentration of colloidal iron is at its highest, viz. immediately after injection. The toxic reactions, however, do not appear until 1—5 hours later, and the UIBC diminishes continuously towards zero during this period (fig. 1 B), even though complete saturation is sometimes found within a few minutes of injection (fig. 1 C).

In the same way the continuous reduction in the UIBC speaks against a photometric error. This also may be expected to have its peak when the light-absorption of the plasma (i. e. the concentration of colloid) is at its greatest. I therefore believe that the change in the UIBC in the doses used here neither can be attributed to precipitation of plasma proteins nor to photometric errors. A rapid physiological fall in the TIBC is unlikely in the light of earlier experience. Samples taken from the same subject at different times of the day, with and without sudden changes in the serum iron, have shown that the TIBC remains constant in contrast with the rapidly fluctuating serum iron

(20, 34). Finally, experiments *in vitro* with addition of saccharated iron oxide show that the genesis of the immediate reduction in the UIBC (table IV) is probably referable to changes in the blood itself and not to extravascular factors. As after injection *in vivo,* an immediate change in the UIBC was obtained *in vitro.* Liberation in the blood of a small fraction of free iron which can become attached to transferrin in the usual way is on the other hand quite likely, as the stability of the saccharated iron oxide diminishes when the pH falls (from 11.0—11.4 in the ampoules to the 7.4 of the blood).

If this presumed change in stability of the saccharated iron oxide is accepted as the explanation of the reduction in the UIBC immediately after injection, the question arises whether the same passive mechanism is sufficient also to explain the continued, successive reduction, or whether an active process is also at work. In order to throw light on this problem the following tests have been carried out.

Three patients received intravenous injections of 100 mg Fe ('Intrafer'). Ten minutes later c. 50 ml of blood was withdrawn and heparinized. A sample was set aside for direct determination of the UIBC. The remainder was rocked in a water bath at 37° C. Samples were taken off at certain intervals, and at the same time fresh specimens were also taken from the patient. The UIBC values were compared. As controls of the ordinary stability of the UIBC under experimental conditions blood samples taken from two of the cases before the injection of iron were placed in a water bath and examined simultaneously. The alteration in the pH of the blood after injection of saccharated iron oxide at the same rate and temperature was, in one trial, 7.46—7.35.

It can be seen from table VI that the UIBC diminishes considerably more *in vivo* than *in vitro.* The levels *in vitro* are in fact so constant that it may be questioned whether further dissociable iron is liberated. To judge from this experiment the reduction in the UIBC *in vivo* cannot be explained by the passive splitting off of iron ions. Other factors to be taken into consideration are either extravascular (e. g. the RES), or intravascular but unable to develop full activity *in vitro* (e. g. enzymes).

I have not been able to demonstrate any direct connection between the toxic reactions following the injection of saccharated iron oxide and saturation of the TIBC. The general reactions seen can be divided into two groups. Those occurring immediately after injection are very similar to the type seen after an ordinary iron salt (see table I), but are considerably slighter. The nearest explanation at hand is a vascular reaction due to rapidly liberated iron ions.

General toxic reactions arising later are of a different type. Bearing in mind the results of these investigations and the diverging clinical picture, I consider the earlier assumption, that these reactions too depend on the exceeding of the TIBC, to be unlikely (table I). They are much more reminiscent of the 'reticulo-endothelial' type of general

Table VI.

Case + Dose	Time in reln. to injectn.	In vivo	In vitro at 37° C	
		UIBC of blood drawn immediately on sampling	UIBC of blood drawn ten mins. after injection	UIBC of blood drawn before injection
G. F. 100 mg Fe	before	240		
	10 min.	180		
	2 hours	100	180	250
	4 hours	50	145	250
	6 hours	50	150	200 (h)
A. E. 100 mg Fe	before	250		
	5 min.	127		
	2 hours	0	100	215
	4 hours	0	(h)	215
B. S. 100 mg Fe	before	425		
	10 min.	320		
	1 hour	260	270	—
	3 hours	160	225	—

UIBC of venous blood repeatedly sampled during the hours after injection of saccharated iron oxide compared with blood in vitro at 37° C. The iron values in serum were 1,660 (G. F.), 1,490 (A. E.) and 1,530 (B. S.) gamma per cent, respectively. (h) = Haemolysis.

reaction. Moreover, an obviously increased frequency of typical toxic signs (table VII) following the injections of saccharated iron oxide was observed in a group of patients (15 b) in whom the RES was probably in some way influenced by simultaneously performed subcutaneous injections of a precipitated toxoid (triple vaccine against diphtheria, tetanus, and pertussis). Exactly corresponding experiments

Table VII.

Children injected with colloidal iron	Number of injections	Mg Fe per kg body-wt. and injection	1–8 hrs slight pyrexia	1–5 hrs Chills, pyrexia, nausea, vomiting	3/4–5 hrs Chills, pyrexia, incipt. shock, pale cyanosis
Immunized (19)	22	8.4 (5—13)	5	9	1
Controls (23)	34	8.1 (5—15)	4	2	1

The frequency of toxic reactions after simultaneous administration of intravenous saccharated iron oxide and subcutaneous toxoid (triple vaccine).

in animals have not been performed to my knowledge. However, in animals given simultaneous intravenous injections of saccharated iron oxide ('Intrafer') and dextran, i. e. two colloidal substances with different affinity for reticulo-endothelial cells, the dextran has been shown to disappear more slowly from the blood stream than is usually the case (25). The RES cells seem to be 'blocked' by phagocytosis of saccharated iron oxide at the expense of the dextran. Possibly a temporary disturbance of the functional state of the RES might also explain the increased frequency of toxic reactions, here observed after simultaneous intravenous iron and subcutaneous vaccination.

Summary

The unsaturated iron-binding capacity and the iron content of serum have been studied after various forms of iron administration. In particular the relationship to the different sets of toxic symptoms encountered has been examined.

1. Five cases with slight toxic reactions following oral administration of iron are described. In three of them the total iron-binding capacity of the serum was not saturated. The symptoms therefore probably cannot be ascribed to a surplus of free iron ions in the blood.

2. Intravenous administration of 0.16 mg or more iron per kg bodyweight of an easily dialysed iron salt resulted 15 minutes later in an iron concentration greater than the total iron-binding capacity. The risk of a general application of the '10 mg intravenous tolerance test' for determination of the total iron-binding capacity is thus stressed. The occurrence of loosely bound iron in the plasma and its power of combining with the plasma proteins is discussed.

3. The unsaturated iron-binding capacity was examined after intravenous administration of saccharated iron oxide. Toxic signs were seen both when an unsaturated iron-binding capacity could still be demonstrated and when it was zero. The same varying values were also found in patients who were symptom-free. The toxic reactions developing 1—5 hours after injection of saccharated iron oxide are thus thought to be unconnected with the exceeding of the total iron-binding capacity. On the other hand, some diminution of the unsaturated iron-binding capacity could be universally shown immediately after injection. The author believes that this fact can be ascribed primarily to a lowering of the stability of the saccharated iron oxide *in vivo*. Thus a small fraction of the iron is liberated as free ions and becomes attached to the transferrin. In the hours following injection a continuous increase in the degree of saturation was usually the case. It is more doubtful whether this can also be put down to alteration in the stability of the saccharate. Experiments *in vitro* make another, possibly more active, intra- or

extra-vascular mechanism appear more likely. Finally, other possible explanations of the toxic reactions following saccharated iron oxide are mentioned. The grounds for increased incidence of toxic symptoms in a group of patients in whom the RES was assumed to be 'overloaded' by simultaneous administration of saccharated iron oxide and diphtheria toxoid are discussed.

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Experimental Investigations on Saccharated Iron Oxide

by

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Since Nissim (1) reported his favourable results with saccharated iron oxide for intravenous use, colloid preparations of this kind have been widely used in the treatment of iron deficiency anaemia. The different preparations have similar but not identical physical, chemical and clinical properties. The investigations below refer mainly to 'Intrafer'[1] (Pharmacia) identical with the English 'Ferrivenin' (Benger). This preparation which has been found to be very polydispersed, has a mean molecular weight of somewhat more than 50 000 (2).

Turbidimetric measurements (2) on dilutions of the preparation (fig. 1) show a satisfactory stability of the complex in a wide range of pH (>11—6). Determinations of the unsaturated iron-binding capacity (UIBC) in serum (3) confirm that the preparation also is stable enough in the blood at a physiological pH. This is evident from the fact that the saturation limit for complex-binding of free iron ions usually is not reached 5—10 minutes after injections of colloidal iron. Only a minor fraction of free iron seems to be liberated at once in the blood. In this way colloidal iron differs from simple iron salt solutions (fig. 2) which instantly combine with the iron-free portion of the iron-binding protein, the transferrin (= siderophilin, $beta_1$-metal-combining globulin). These facts explain why iron concentrations in plasma far exceeding the total iron-binding capacity (TIBC) of human serum (300—360) are usually well tolerated when colloidal iron preparations are used.

[1] The iron preparation used was kindly supplied by Pharmacia Ltd., Manufacturing Chemists in Uppsala, Sweden.

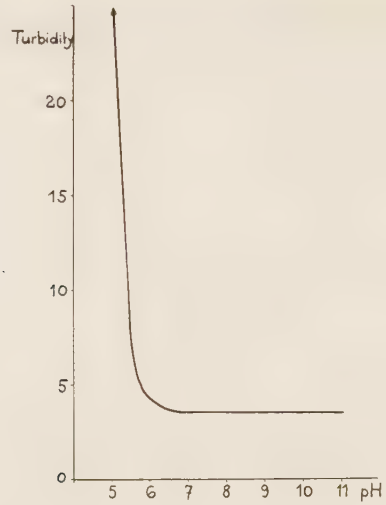

Fig. 1. Turbidimetric measurement (2) of the stability at different pH of the saccharated iron oxide used ('Intrafer').

1. Experiments with dialysis at varying pH

Some experiments with dialysis[2] at varying pH were performed (table I) to determine the amount of dialysable iron (ionized or low molecular saccharated iron oxide) compared with the amount of iron apparently attached to the high molecular part of the preparation used. The dialyses were made against distilled water, the first with the

Table 1.

Dialysis no.	pH	High molecular part, % of initial dry substance	Iron content, % of the high mol. non-dialysable part	Dialysable iron gamma/ml 'Intrafer'
I	11.0	11.3	60.0	3.1
II	7.4	12.1	62.0	2.2
III	5.08	13.0	55.8	28.0

Dialysis at varying pH of the commercial saccharated iron oxide ('Intrafer', containing 20 mg Fe per ml) against distilled water over a period of 3 days. The relation between the initial volumes inside and outside was about 25/300. The amount of the total dried substance as well as of the high molecular portion after dialysis were determined by weight.

[2] The dialyses were kindly performed by lic. K. Granath.

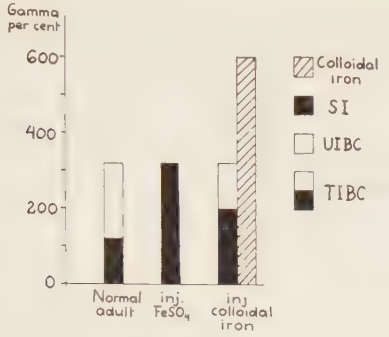

Fig. 2. Shematic figure illustrating the difference between dialysable iron and colloidal iron in their relation to the iron-binding protein in the blood. SI = serum iron, UIBC = unsaturated iron-binding capacity, TIBC = total iron-binding capacity.

undiluted preparation as the inner medium. In the others, the preparation was acidified to a required pH by adding sulphuric acid. The first experiment was thus performed at the pH of the commercial preparation, the second at the physiological pH of the blood, and the third at a pH, where the preparation is unstable as shown by the turbidimetric determinations.

It appears, as is shown in table I, that a notable amount of iron was found only in the third type of dialysis. At a physiological pH practically all of the iron seems to be incorporated in the small high molecular fraction of the preparation. The percentage iron content of this portion as measured here is in good agreement with the value calculated from the formula (4.5), according to which the saccharated iron oxide contains 16 Fe-atoms per molecule saccharate, i. e., 58.4 per cent of iron.

These findings confirm the excellent stability of the concentrated preparation *in vitro*. One cannot draw any conclusions, however, as to whether it is as stable when diluted in the circulating blood with its complex properties. A certain decrease of the unsaturated iron-binding capacity found already 5—10 minutes after injection (3) might well signify an immediate liberation of a small fraction of free iron. If this is in fact true, the fraction must, however, be larger than the very small amounts which dialysed *in vitro*.

2. Photometric analysis of saccharated iron oxide after acid hydrolysis

The acid soluble iron in serum is quantitatively split off from its iron-protein complex after moderate acidification. By determination of

the serum iron according to Heilmeyer & Plötner (6) with the modified method of Vahlquist (7), hydrochloric acid and trichloracetic acid are added in concentrations of 4 and 8 per cent. In this way the native serum iron is liberated in a free form. To elucidate the extent to which iron in colloidal form is recovered with the usual serum iron method, analyses were performed on water dilutions of 'Intrafer' and another colloidal preparation, 'Ferronascin' (Roche), which has quite a different chemical constitution (natrium-di (α_1 γ-dioxy-B_1B-dimethyl-butyrato) ferrat (III)).

Table II.

Preparation	Gamma per cent Fe	
	Expected	Found
Intrafer ..... } Ferrivenin ..... }	200	144
Ferronascin	200	190

Yield of iron from two different commercial preparations of colloidal iron.

It appears, as is shown in table II that the iron of 'Intrafer' is recovered only to some 65—75 per cent of the calculated value, while 'Ferronascin' iron gives a yield of 95 per cent. A similar difference but a much smaller amount of the calculated iron concentration was found in serum 5 minutes after injections of the two preparations to healthy volunteers (fig. 3). The results suggest that the saccharated iron oxide is a more stable fraction than the other colloidal iron used. Further, the low values obtained after injection *in vivo* point either to a worse yield of colloidal iron in whole blood or to its rapid disappearance from the blood immediately after the injection. The last assumption seems unprobable, as the preparation is a high molecular colloid which is phagocytosed by reticulo-endothelial cells and therefore leaves the blood successively.

The experiments tabulated in figure 4 and table III were performed to elucidate the effect on saccharated iron oxide of extended time of reaction for hydrochloric acid, increased acidity, and enhanced temperature during the time of reaction.

It is seen from figure 4 that the percentage yield of iron increases as the time of the reaction is extended. Most of the increase occurs to the first hour. Thereafter it is slow for the next six hours. After this time, stability seems to be reached. The maximal percentage yield of iron is less than 80 per cent in serum dilutions. The changes are similar to those of Overkamp (8) who worked with other preparations of saccharated iron oxide. His final yield was, however, 100 per cent.

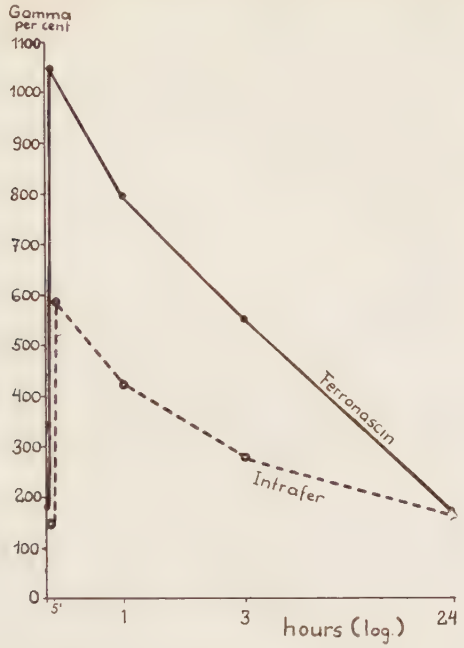

Fig. 3. The iron concentration in the serum after intravenous injections of 'Intrafer' and 'Ferronascin' into the same three healthy adult men, each receiving 0.5 mg Fe per kg bodyweight in both of the injections. The curves refer to the mean values. The iron analyses were performed with the usual serum iron technique.

Table III.

Diluent and Conc. of Hydrochloric acid	Per cent iron recovered			Number of experiments[1]
	At 20° C	At 60° C		
	15 min.	15 min.	60 min.	
water..... 24 % HCl	95	95	99	3
water..... 12 % »	86	98	97	3
water..... 8 % »	78	93	93	3
water + trichlor-acetic acid .. 4 % »	74	89	94	3
serum + trichlor-acetic acid .. 4 % »	43	71	97	5

Determinations of the iron yield from water and serum dilutions of saccharated iron oxide in a concentration of 0.2 mg Fe per 100 ml.

[1] Double determinations were always made.

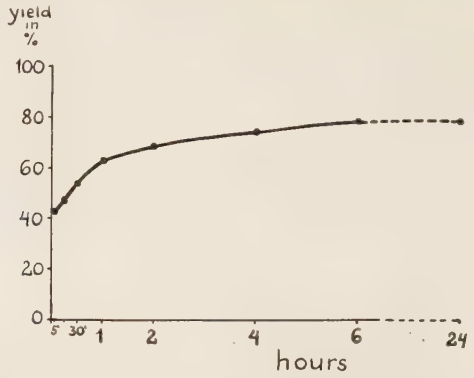

Fig. 4. Percentage yield of iron at varying reaction time for the hydrochloric acid. The determinations were made on serum dilutions containing 200 gamma per cent colloidal iron. The usual serum iron technique was used.

A successively increased acidity liberates an increasing amount of iron from the complex binding. From a reaction mixture with a six fold increased concentration of hydrochloric acid, one gets 95 per cent of the calculated iron in a water dilution of 'Intrafer'. Less is regained from serum dilutions where only 40—45 per cent can be found with the usual serum iron technique. It is possible that some of the colloid iron is precipitated with the proteins. This assumption is supported by the fact that a considerable amount of iron was left in the precipitate. This was shown by re-adding trichloracetic acid and hydrochloric acid to the precipitate after the free iron salt solution had been taken off for usual analysis.

If the temperature of the reaction mixtures is increased to 60° C, there is a far better yield of iron both from water and serum dilutions. After 15 minutes it is 89 and 73 per cent respectively. When the time of reaction is prolonged to one hour the corresponding yield was some 95 per cent from both media.

Summing up it may be said that serum iron determinations with the usual technique yield about 70—75 per cent of the iron in water dilutions of saccharated iron oxide and 40—45 per cent in serum dilutions. If the reaction mixture is kept for an hour at 60° C practically all of the iron is generally recovered from both of the test solutions.

3. The behaviour of saccharated iron oxide after intravenous injection

The next step was to analyse sera taken from patients, who had received 50—100 mg intravenous iron as 'Intrafer' 5—10 minutes pre-

Table IV.

Blood-iron mixture obtained	Number of cases	Per cent iron recovered of the calculated		mg Fe injected/added per 100 cc plasma
		at 20° C 15 min.	at 60° C 60 min.	
in vivo (whole blood)	10	43 (32.5—53.6)	62 (47.3—84.3)	2—8
in vitro (whole blood)	4	35—45	50—60	4
in vitro (serum)	4	50—60	80—90	2—4

Percentage yield of iron from saccharated iron oxide, injected or added to serum in concentrations corresponding to those found after therapeutic doses.

viously. After injections of this magnitude the iron concentration in serum amounts to some 500—2 000 gamma per cent as measured with the usual serum iron technique. To get iron concentrations suitable for analysis, serum was diluted with iron-free water 6—12 times its own volume. As comparison four samples were used, where saccharated iron oxide in comparable amount was added in *vitro*, just when the venous blood was drawn. In this way as similar *in vitro* conditions as possible were obtained.

As shown in table IV the yield is about 40 per cent of the calculated value, in both the in *vivo* and in *vitro* preparations, with the usual technique. However, as the sample approaches that of a water dilution a better yield is to be expected. This is supported by the fact that more iron was recovered from comparable serum-water dilutions with corresponding amount of iron added. Probably some of the colloidal iron is not present in the serum as even the yield at 1 hour and 60° C was no more than 60 per cent of the calculated value.

The lower values obtained on whole blood samples are surprising. There is, however, no reason to believe that the iron leaves the blood *in vivo* as the yield was quite as poor, when iron was added *in vitro*. The iron ought to be left in the blood. Our hypothesis is that part of the iron is to be found among the packed red cells after coagulation and centrifugation of the sample. This may explain why not more than maximum 60 per cent was recovered from whole blood.

Even during the first few hours after injection the iron content of the serum decreases rapidly (figs. 3 and 5) as the colloidal iron is being taken up by reticulo-endothelial cells. 24 hours after injection the iron concentration is usually back to normal, but increased values are found after large doses (10). With the usual serum iron technique it is impossible to say if part of the iron still exists in a complex form. Determination of the unsaturated iron-binding capacity may give a

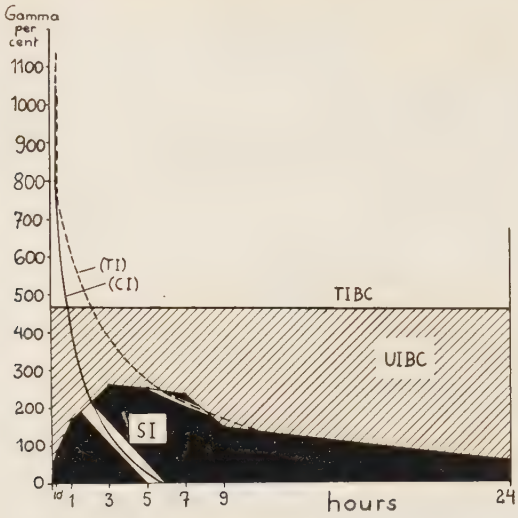

Fig. 5. The rate of disappearance from the blood of 50 mg iron as saccharated iron oxide. The values refer to a 27 year old student nurse with iron deficiency anaemia. The total iron-binding capacity (TIBC) was determined before and 24 hours after the injection. It was assumed to be unaltered between these two measurements. The changes in total iron concentration (TI) and unsaturated iron-binding capacity (UIBC) were determined continuously. The remainder of the values have been calculated from these. (CI) = calculated amount of colloidal iron.

hint. In the physiologic state this capacity added to the serum iron value constitutes the total iron-binding capacity (TIBC) of the serum (fig. 2). In contrast to the fluctuating serum iron, it remains from day to day at a fairly constant level in healthy subjects. 24 hours after injections of saccharated iron oxide, an increased 'total iron-binding capacity' is sometimes found, especially after closely repeated injections. This might be explained by the presence in the blood of a small remaining amount of colloidal iron which is erroneously added to the real total iron-binding capacity (fig. 6).

On the contrary, the saccharated iron oxide seems to disappear more rapidly from tre blood of previously untreated patients with iron deficiency. Thus the serum of the patient in fig. 5 was apparently free from colloidal iron 5 hours after the injection. The dose was, however, only 50 mg in this case.

Summary

1. Dialysis confirmed the results of turbidimetric determinations that the saccharated iron oxide used ('Intrafer' = 'Ferrivenin') is stable *in*

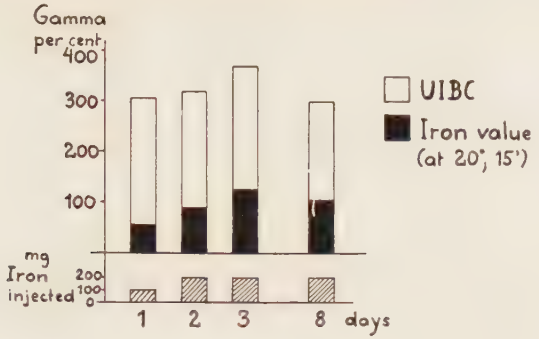

Fig. 6. The iron content and the unsaturated iron-binding capacity in serum 24 hours after repeated intravenous injections of 100—200 mg Fe as 'Intrafer'.

vitro in a wide range of pH (> 11—6). The iron content of the dried non-dialysable substance amounts to some 60 per cent thus bound in a high molecular form.

2. With the usual serum iron technique only about 40—45 per cent of the colloidal iron in serum dilutions made *in vitro* was recovered. There was a far better yield either by increasing the acidity, or extending the time of reaction for hydrochloric acid, or enhancing the temperature during the time of reaction. Practically all of the iron was recovered at a reaction time of one hour and a reaction temperature of 60° C.

3. Five to ten minutes after injections of saccharated iron oxide the yield from whole blood samples with the above-mentioned technique was not more than some 60 per cent of the calculated value. As about the same values were obtained after adding colloidal iron to whole blood *in vitro,* the authors believe that the rest of the iron is not extravasated but is trapped among the red cells.

4. The content of colloidal iron in the blood decreased rapidly during the hours following injection. The iron concentration of the serum was usually normal 24 hours after the injection of 50—200 mg of iron in adults. Repeated simultaneous determinations of the iron content and the unsaturated iron-binding capacity of the serum showed that the colloidal iron sometimes had left the blood even after 5—6 hours, while in other cases a small fraction still remained in the blood after 24 hours.

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Särtryck ur Nordisk Medicin 1953;49:252

Antikroppsbildningen hos barn efter stora engångsdoser järnoxidsackarat intravenöst.

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(Från Pediatriska kliniken i Uppsala; chef: professor Bo Vahlquist.)

Antikroppsbildningen efter »blockad» av det retikuloendoteliala systemet (RES) undersöktes och diskuterades livligt under 1920- och 1930-talen. Flera författare ansåg sig ha påvisat en säker hämning, medan andra förnekade detta. Uttömmande sammanställningar av de divergerande uppfattningarna och resultaten har lämnats av Cannon & al.[3], [17], [15]. Under de senaste åren har problemen rörande antikroppsbildningen efter belastning av RES ånyo aktualiserats, sedan kolloidala föreningar som dextran, periston och järnoxidsackarat fått en omfattande spridning. Flertalet kolloider, som införes i blodbanan, ger nämligen i stora doser en mer eller mindre uttalad »blockad» av RES.

Långt innan järnoxidsackarat började användas som terapeuticum vid järnbrist, visste man, att det var en av de bäst blockerande, kolloidala substanserna[4]. Som sådant kom det också till användning vid djurexperimentella undersökningar över antikroppsbildningens relation till RES[3], [17], [15], [21], [20]. Nyligen anser sig Gabrieli[9] ha visat, att även nutida järnoxidsackarat för kliniskt bruk har blockerande egenskaper. Han fann dessutom belägg för en temporärt hämmad enzymatisk aktivitet som sekundär följd till blockad av RES[10]. Analogt fanns även anledning misstänka en rubbning av antikroppsbildningen[18], [11].

Det är kliniskt viktigt att utröna i vad mån antikroppsbildningen påverkas av järnoxidsackarat i terapeutiska doser. De barn, som behöver behandlas med intravenöst järn, d.v.s. i första hand coeliakier och ulcerösa coliter med

järnbrist[12], måste nämligen redan primärt betraktas som mindre resistenta mot infektioner.

Ett utmärkt tillfälle att studera problemet gavs, då barnen på ett vårdhem för sinnesslöa skulle skyddsympas mot difteri (boosterdos). Trots en tillfredsställande kost hade nämligen ett flertal av dessa barn lätta till måttliga järnbristanämier. Järnbehandling var alltså motiverad.

Material och metodik. 16 barn i åldern 3—12 år, som samtliga ympats mot difteri ett halvt till ett år tidigare, fick 5,0—10,7 mg Fe per kg kroppsvikt i form av en intravenös injektion järnoxidsackarat (intrafer, Pharmacia). Omedelbart efter järninjektionen injicerades subkutant i fossa supra spinam difterianatoxin[6] i form av 1 ml trippelvaccin[7] (Statens Bakteriologiska Laboratorium). Det använda trippelvaccinet innehöll 12,5 flockningsenheter difterianatoxin, 7,5 flockningsenheter stelkrampsanatoxin samt 15 miljarder kikhostebakterier per ml. Blod för titreringen på antitoxintitern mot difteri togs före injektionerna, 3 tim. efter samt slutligen 1 månad senare. Som kontroller användes barn, som enbart fick 1 ml trippelvaccin. Mot båda grupperna kan invändas, att de är heterogena ur ålderssynpunkt. Detta faktum torde dock inte spela någon roll, då förmågan att bilda antitoxin mot difteri är likvärdig i alla åldrar efter första levnadskvartalet[26]. Utöver de två större grupperna gavs dessutom ytterligare 5 barn 10 mg Fe per kg kroppsvikt samt 12,5 enheter difterianatoxin av kortverkande typ, alltså ej adsorberat som i trippelvaccinet. Dessa barn följdes med antitoxintitreringar efter 1, 2 och 3 veckor för att se, om vaccin av kortvarig effekt skulle kunna avslöja en eventuell, snabbt övergående funktionell »blockad», d.v.s. om titerstegringen på en sådan boosterdos blev prompt, fördröjd eller hävd.

Antitoxintitreringarna gjordes av Statens Bakteriologiska Laboratorium enligt Jenssens metod[16].

Resultat.

Tabell I visar, att antitoxintitern steg genomgående såväl i det järnbehandlade och ympade som i det enbart ympade kontrollmaterialet. Kvantitativt var stegringen tillfredsställande, i synnerhet som flera av barnen redan före boosterdosen hade höga titrar. Någon kvantitativ skillnad mellan de båda materialen kunde ej med säkerhet påvisas, vilket framgår av tabell II. Variationen av järndosens storlek inom de använda gränserna, 5—11 mg per kg kroppsvikt, tycktes inte spela någon roll. Antitoxintitern 3 tim. efter järninjektionen var hos 10 patienter av 14 identisk eller praktiskt taget identisk med utgångsvärdet. I 2 fall

TABLE I A. *Treated.*

Case no.	Age years sex	Mg Fe per kg b.-wt.	Antitoxin level, units per cc. I before immunization	Antitoxin level, units per cc. II 1 month after immunization	Quotient II/I
1	4¾ ♂	10.3	0.36	>288	800
2	6 ♂	10.8	2.7	c:a 288	107
3	4 ♂	10.0	2.1	224	107
4	12½ ♀	5.1	0.56	56	100
5	9 ♂	5.0	2.1	168	80
6	9¾ ♀	6.6	7.0	168	24
7	3¼ ♂	7.7	3.6	36	10
8	12 ♀	5.2	2.4	24	10
9	9½ ♂	7.5	12.0	96	8
10	5½ ♀	10.7	32.0	256	8
11	8½ ♂	10.4	2.8	21	7.5
12	6¾ ♂	5.3	6.3	27	4.3
13	7½ ♂	10.4	18.0	72	4.0
14	6½ ♂	5.0	16.0	64	4.0
15	10¼ ♀	7.0	12.0	48	4.0
16	11¼ ♀	5.1	1.6	3.2	2.0

Table I. Antitoxin levels before and after immunization, A. in the iron-injected and vaccinated children, B. in the vaccinated controls.

var 3-timmarstitern hälften så stor, i de 2 resterande omkring dubbelt så stor som utgångstitern.

Fig. 1 visar, att ett kortverkande difterianatoxin av samma styrka som det alunprepiciterade gav en motsva-

TABLE I B. *Controls.*

Case no.	Age years sex	Antitoxin level, units per cc. I before immunization	Antitoxin level, units per cc. II 1 month after immunization	Quotient II/I
1	11 ♀	0.6	96	160
2	10 ♂	0.6	72	120
3	7¼ ♂	1.2	72	60
4	3¾ ♂	0.4	20	50
5	6½ ♂	0.5	20	40
6	2 ♂	0.025	0.90	36
7	13¾ ♀	5.0	80	16
8	2¼ ♀	10.0	80	8
9	8 ♀	9.0	54	6
10	2 ♀	2.4	9	3.8
11	8¼ ♂	15.0	40	2.7
12	13¾ ♀	11.0	28	2.6
13	5 ♀	24.0	48	2.0
14	19 ♀	14.0	21	1.5

TABLE II.

	A.m.q.	I.m.q.
Iron-injected	21.3	9
Controls	10.7	12

Average antitoxin response in the iron-treated compared with the controls one month after subcutaneous vaccination with diphtheria toxoid (booster-dose). The increase is expressed in quotients (individual median quotient = i.m.q., and average median quotient = a.m.q., i.e. median titre after/before vaccination).

rande stegring av antitoxintitern under den första månaden. Mestparten av denna stegring skedde redan under första veckan efter ympningen.

Som bifynd vid undersökningen noterades 1—5 tim. efter de båda injektionerna en hög frekvens toxiska symtom (tabell III) av den typ, som man annars blott ser i ett mindre antal fall efter järnoxidsackarat intravenöst, nämligen feber, frysningar, illamående, kräkningar och i undantagsfall lätt shock[12, 13]. Järnoxidsackarat från endast

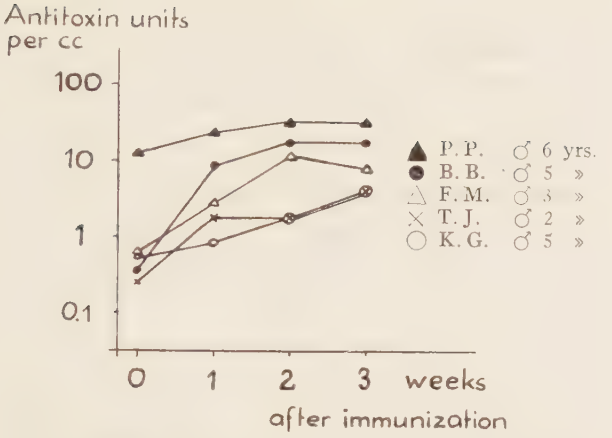


Fig. 1. Antitoxin response to vaccination with non-adsorbed diphthera toxoid (12,5 AU) after intravenous injection of saccharated iron oxide (10 mg Fe per kg b.-wt.).

två olika charger användes och gavs omväxlande i de båda grupperna. Skillnaden i intoxikationsfrekvensen kan därför knappast förklaras genom olikheter i preparatets framställning.

Diskussion. Exakt var och hur antikroppsbildningen sker är fortfarande oklart[14]. Diverse reticuloendoteliala element har emellertid angivits vara av betydelse[5, 1, 8, 14]. Under-

TABLE III.

Children injected with colloidal iron	Number of injections	Mg Fe per kg body-wt and injection	1—8 hrs slight pyrexia	1—5 hrs chills, pyrexia, nausea, vomiting	¾—5 hrs chills, pyrexia, incip. shock, pale cyanosis
Vaccinated (19)	22	8.4 (5—13)	5	9	1
Controls (23)	34	8.1 (5—15)	4	2	1

The frequency of toxic reactions after simultaneous administration of intravenous saccharated iron oxide and subcutaneous toxoid (triple-vaccine).

sökningar över antikroppsbildningen hos människa efter intravenös tillförsel av järnoxidsackarat har veterligt ej utförts tidigare. På djur har dock visats, att aktiva immuniteten och antikroppsbildningen mot exempelvis pneumokokker kunde hämmas avsevärt efter massiva doser kolloidalt järn[21], [20], medan mindre doser kolloid däremot inte sällan gav en stegrad antikroppsbildning[15], [21].

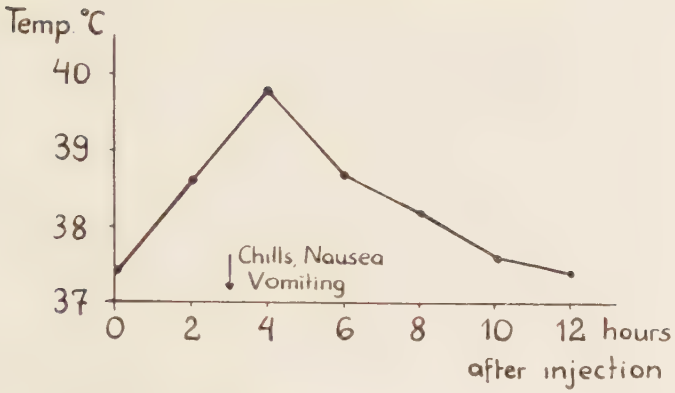
Jungeblut & Berlot[18] fann en störning i antikroppsbildningen av en tredje typ. Efter sannolik blockad av RES med india ink och samtidig ympning mot difteri erhöll de en fördröjd men för övrigt tillfredsställande stegring av antitoxintitern.

Gabrielli[9] studerade *på råttor* kolloidala järnpreparats blockerande effekt på RES medelst en radioaktiv testkolloid. En partiell »blockad» erhölls efter injektion av intrafer (ferrivenin) i en dos om lägst 20 mg Fe per kg kroppsvikt. »Blockaden» kunde påvisas redan efter 1 tim., nådde sitt högsta uppmätta värde efter 6 tim. samt var efter ett dygn på tillbakagång.

Barnen i det här behandlade materialet fick 5—11 mg Fe per kg kroppsvikt intravenöst i en engångsdos, d.v.s. samma administrationssätt och i stort sett samma dos som författaren tidigare använt sig av vid intravenös järnbehandling av barn[12]. Av resultaten framgår, att antitoxinbildningen efter boosterympning mot difteri uppenbarligen varken var sämre eller bättre i det behandlade än i det obehandlade materialet. Någon fördröjning av titerstegringen, som kan ha praktiskt intresse, förelåg heller inte, eftersom adekvata svar på antigenet erhölls inom första veckan.

Medan man av tekniska och psykologiska skäl med fördel ger barn intravenöst järn i form av stora engångsdoser, får vuxna vanligen upprepade, mindre injektioner. Om effekten på antitoxinbildningen därvid blir en annan har ej undersökts. Teoretiskt är det dock tänkbart. Djurexperimentella undersökningar tyder nämligen på en effektivare »blockad» av RES efter ett sådant administrationssätt[22].

Den höga frekvensen toxiska symtom i den behandlade gruppen är anmärkningsvärd (tabell III). Efter enbart trippelvaccinering med samma ympvärde som det här använda fann Ericsson, Hesselvik & Vahlquist[7] i 28 % en temperaturstegring mellan 38—39° C under det första dygnet. Därutöver noterades inga andra allmänsymtom än



Case J. Å. P. 4 years
10,0 mg Fe/kg b.wt.

Fig. 2. Characteristic febrile reaction during the hours following an injection of saccharated iron oxide.

grinighet och irritation. I författarens ympade kontrollmaterial noterades snarast än mindre bireaktioner. Efter intrafer (ferrivenin) finner man likaså av och till övergående temperaturstegringar under de första 6—8 tim. (se fig. 2). Mindre vanliga är påtagliga toxiska reaktioner med hög feber, frysningar och kräkningar samt i enstaka undantagsfall shocksymtom. Med samma järnmängder, som använts här, har författaren tidigare noterat sådana symtom efter 3 injektioner av 34. Som synes av tabell III ger däremot kombinationen järnoxidsackarat intravenöst och trippelvaccin subkutant en hög frekvens toxiska reaktioner av den just omtalade typen.

Bakgrunden till de toxiska reaktionerna efter järnoxidsackarat är ännu outredd. Enligt vissa författares mening[2, 19] uppstår toxiska symtom endast då blodplasmats järntransporterande globulin, transferrinet, överbelastas. Denna teori anser sig författaren ha vederlagt i annat sammanhang[13]. Andra menar att symtomen orsakas av komplexa järn-äggviteprecipitat[25, 24]. Slutligen har också den kliniska likheten med reticuloendoteliala reaktioner påpekats[23].

Övertygande belägg för eller emot de två sistnämnda teorierna har hittills icke lämnats. Enligt de här gjorda

antitoxinstudierna kan man endast säga, att någon utfällning eller inaktivering av immunglobulin inte förefaller trolig, eftersom titrarna förblev oförändrade 3 tim. efter järninjektionerna, d.v.s. då de toxiska reaktionerna har sitt genomsnittliga maximum.

De toxiska reaktionernas kliniska bild och den ökade frekvensen intoxikationer i det här behandlade materialet är enligt författarens mening bäst förenliga med en reticuloendotelial överbelastning. Järnet, som är i kolloidal form, och difterianatoxinet, som är ett antigen, skall båda på kort tid tagas om hand av RES, som på så vis blir dubbelbelastat. Direkt analoga, experimentella undersökningar har veterligt ej gjorts. Däremot vet man, att samtidig intravenös belastning med järnoxidsackarat och radioaktivt chromiumfosfat[9] eller dextran[22] ger en förlångsammad upptagning av de båda sistnämnda testkolloiderna ur blodbanan. Möjligen skulle en temporär nedsättning av RES' funktionella kapacitet även kunna vara bakgrunden till den ökade intoxikationsfrekvensen vid samtidig tillförsel av järnoxidsackarat och difterianatoxin. Säkerligen bör man dock akta sig för att draga några vittgående paralleller mellan dessa sistnämnda kliniska iakttagelser och de toxiska reaktionernas genes.

LITTERATUR.

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The effect of large single doses of saccharated iron oxide on antibody production in children.

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The production of antitoxin in children was studied after simultaneous administration of diphtheria toxid (booster dose) and intravenous saccharated iron oxide (5—11 mg Fe per kg bodyweight in a single dose). The antitoxin response was quite satisfactory compared with that of a control group. Thus colloidal iron in the doses used seems to have no functional »blocking» effect on the antibody production of the reticulo-endothelial cells.

Toxic reactions of the type seen after intravenous saccharated iron oxide were noted here in an obviously increased frequency compared with children only injected with iron. This fact might have something to do with an overloading of the reticulo-endothelial system.

6. XII. 1952.

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Uppsala 1953. Almqvist & Wiksells Boktryckeri AB 53:173